

# NOVEL PROTEINS AND NUCLEIC ACIDS ENCODING SAME

## RELATED APPLICATIONS

This application claims priority to U.S. provisional application Serial No. 60/171,329,  
5 filed December 21, 1999, which is incorporated herein by reference in its entirety.

## FIELD OF THE INVENTION

The invention generally relates to nucleic acids and polypeptides encoded therefrom.  
More specifically, the invention relates to nucleic acids encoding novel polypeptides, as well as  
vectors, host cells, antibodies, and recombinant methods for producing these nucleic acids and  
10 polypeptides.

## SUMMARY OF THE INVENTION

The invention is based in part upon the discovery of novel nucleic acid sequences  
encoding novel polypeptides. Nucleic acids encoding the polypeptides disclosed in the  
invention, and derivatives and fragments thereof, will hereinafter be collectively designated as  
15 "FCTRX" nucleic acid or polypeptide sequences.

In one aspect, the invention provides an isolated FCTRX nucleic acid molecule encoding  
a FCTRX polypeptide that includes a nucleic acid sequence that has identity to the nucleic acids  
disclosed in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29. In some  
embodiments, the FCTRX nucleic acid molecule can hybridize under stringent conditions to a  
20 nucleic acid sequence complementary to a nucleic acid molecule that includes a protein-coding  
sequence of a FCTRX nucleic acid sequence. The invention also includes an isolated nucleic  
acid that encodes a FCTRX polypeptide, or a fragment, homolog, analog or derivative thereof.  
For example, the nucleic acid can encode a polypeptide at least 80% identical to a polypeptide  
comprising the amino acid sequences of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24,  
25 26, 28 and 30. The nucleic acid can be, for example, a genomic DNA fragment or a cDNA  
molecule that includes the nucleic acid sequence of any of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15,  
17, 19, 21, 23, 25, 27, and 29.

Also included in the invention is an oligonucleotide, *e.g.*, an oligonucleotide which  
includes at least 6 contiguous nucleotides of a FCTRX nucleic acid (*e.g.*, SEQ ID NOS:1, 3, 5,  
30 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29) or a complement of said oligonucleotide.

Also included in the invention are substantially purified FCTR<sub>X</sub> polypeptides (SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30). In some embodiments, the FCTR<sub>X</sub> polypeptides include an amino acid sequence that is substantially identical to the amino acid sequence of a human FCTR<sub>X</sub> polypeptide.

5 The invention also features antibodies that immunoselectively-binds to FCTR<sub>X</sub> polypeptides.

In another aspect, the invention includes pharmaceutical compositions which include therapeutically- or prophylactically-effective amounts of a therapeutic and a pharmaceutically-acceptable carrier. The therapeutic can be, *e.g.*, a FCTR<sub>X</sub> nucleic acid, a FCTR<sub>X</sub> polypeptide, 10 or an antibody specific for a FCTR<sub>X</sub> polypeptide. In a further aspect, the invention includes, in one or more containers, a therapeutically- or prophylactically-effective amount of this pharmaceutical composition.

In a further aspect, the invention includes a method of producing a polypeptide by culturing a cell that includes a FCTR<sub>X</sub> nucleic acid, under conditions allowing for expression of the FCTR<sub>X</sub> polypeptide encoded by the DNA. If desired, the FCTR<sub>X</sub> polypeptide can then be 15 recovered.

In another aspect, the invention includes a method of detecting the presence of a FCTR<sub>X</sub> polypeptide in a sample. In the method, a sample is contacted with a compound that selectively binds to the polypeptide under conditions allowing for formation of a complex between the polypeptide and the compound. The complex is detected, if present, thereby identifying the 20 FCTR<sub>X</sub> polypeptide within the sample.

The invention also includes methods to identify specific cell or tissue types based on their expression of a FCTR<sub>X</sub>.

Also included in the invention is a method of detecting the presence of a FCTR<sub>X</sub> nucleic acid molecule in a sample by contacting the sample with a FCTR<sub>X</sub> nucleic acid probe or primer, 25 and detecting whether the nucleic acid probe or primer bound to a FCTR<sub>X</sub> nucleic acid molecule in the sample.

In a further aspect, the invention provides a method for modulating the activity of a FCTR<sub>X</sub> polypeptide by contacting a cell sample that includes the FCTR<sub>X</sub> polypeptide with a 30 compound that binds to the FCTR<sub>X</sub> polypeptide in an amount sufficient to modulate the activity of said polypeptide. The compound can be, *e.g.*, a small molecule, such as a nucleic acid, peptide, polypeptide, peptidomimetic, carbohydrate, lipid or other organic (carbon containing) or inorganic molecule, as further described herein.

Also within the scope of the invention is the use of a Therapeutic in the manufacture of a 35 medicament for treating or preventing disorders or syndromes including, *e.g.*, cancer,

neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders. The Therapeutic can be, *e.g.*, a FCTR<sub>X</sub> nucleic acid, a FCTR<sub>X</sub> polypeptide, or a FCTR<sub>X</sub>-specific antibody, or biologically-active derivatives or fragments thereof.

5 The invention further includes a method for screening for a modulator of disorders or syndromes including, *e.g.*, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders. The method includes contacting a test compound with a FCTR<sub>X</sub> polypeptide and determining if the test compound binds to said FCTR<sub>X</sub> polypeptide. Binding of the test compound to the FCTR<sub>X</sub> polypeptide  
10 indicates the test compound is a modulator of activity, or of latency or predisposition to the aforementioned disorders or syndromes.

Also within the scope of the invention is a method for screening for a modulator of activity, or of latency or predisposition to an disorders or syndromes including, *e.g.*, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and  
15 hematopoietic disorders, by administering a test compound to a test animal at increased risk for the aforementioned disorders or syndromes. The test animal expresses a recombinant polypeptide encoded by a FCTR<sub>X</sub> nucleic acid. Expression or activity of FCTR<sub>X</sub> polypeptide is then measured in the test animal, as is expression or activity of the protein in a control animal which recombinantly-expresses FCTR<sub>X</sub> polypeptide and is not at increased risk for the disorder  
20 or syndrome. Next, the expression of FCTR<sub>X</sub> polypeptide in both the test animal and the control animal is compared. A change in the activity of FCTR<sub>X</sub> polypeptide in the test animal relative to the control animal indicates the test compound is a modulator of latency of the disorder or syndrome.

In yet another aspect, the invention includes a method for determining the presence of or  
25 predisposition to a disease associated with altered levels of a FCTR<sub>X</sub> polypeptide, a FCTR<sub>X</sub> nucleic acid, or both, in a subject (*e.g.*, a human subject). The method includes measuring the amount of the FCTR<sub>X</sub> polypeptide in a test sample from the subject and comparing the amount of the polypeptide in the test sample to the amount of the FCTR<sub>X</sub> polypeptide present in a control sample. An alteration in the level of the FCTR<sub>X</sub> polypeptide in the test sample as  
30 compared to the control sample indicates the presence of or predisposition to a disease in the subject. Preferably, the predisposition includes, *e.g.*, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders. Also, the expression levels of the new polypeptides of the invention can be used in a method to screen for various cancers.

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In a further aspect, the invention includes a method of treating or preventing a pathological condition associated with a disorder in a mammal by administering to the subject a FCTRX polypeptide, a FCTRX nucleic acid, or a FCTRX-specific antibody to a subject (*e.g.*, a human subject), in an amount sufficient to alleviate or prevent the pathological condition. In preferred embodiments, the disorder, includes, *e.g.*, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

In yet another aspect, the invention can be used in a method to identify the cellular receptors and downstream effectors of the invention by any one of a number of techniques commonly employed in the art. These include but are not limited to the two-hybrid system, affinity purification, co-precipitation with antibodies or other specific-interacting molecules.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

## DETAILED DESCRIPTION

The invention is based, in part, upon the discovery of novel nucleic acid sequences that encode novel polypeptides. The novel nucleic acids and their encoded polypeptides are referred to individually as FCTR1, FCTR2, FCTR3, FCTR4, FCTR5, FCTR6, FCTR7, FCTR8, FCTR9, FCTR10, FCTR11, FCTR12, FCTR13, and FCTR14. The nucleic acids, and their encoded polypeptides, are collectively designated herein as "FCTRX".

The novel FCTRX nucleic acids of the invention include the nucleic acids whose sequences are provided in Tables 1A, 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A, 10A, 11A, 12A, 13A, and 14A, inclusive ("Tables 1A - 14A"), or a fragment thereof. The invention also includes a mutant or variant FCTRX nucleic acid, any of whose bases may be changed from the corresponding base shown in Tables 1A - 14A while still encoding a protein that maintains the activities and physiological functions of the FCTRX protein fragment, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including complementary nucleic acid fragments. The invention

additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject. In the mutant or variant nucleic acids, and their complements, up to 20% or more of the bases may be so changed.

The novel FCTR<sub>X</sub> proteins of the invention include the protein fragments whose sequences are provided in Tables 1B, 2B, 3B, 4B, 5B, 6B, 7B, 8B, 9B, 10B, 11B, 12B, 13B, and 14B, inclusive ("Tables 1B - 14B"). The invention also includes a FCTR<sub>X</sub> mutant or variant protein, any of whose residues may be changed from the corresponding residue shown in Tables 1B - 14B while still encoding a protein that maintains its native activities and physiological functions, or a functional fragment thereof. In the mutant or variant FCTR<sub>X</sub> protein, up to 20% or more of the residues may be so changed. The invention further encompasses antibodies and antibody fragments, such as F<sub>ab</sub> or (F<sub>ab</sub>)<sub>2</sub>, that bind immunospecifically to any of the FCTR<sub>X</sub> proteins of the invention.

#### **FCTR1 (AL031943\_A)**

The novel FCTR1 nucleic acid encoding a C-terminal fragment of a novel FCTR1 protein is shown in Table 1A. A "TAA" stop codon was identified at the 3' end indicating that this sequence is a coding sequence. The stop codon is shown in bold letters. This sequence originates in chromosome 6. No ATG start codon was found, indicating that the cDNA extends 5' of the disclosed sequence in Table 1A.

**Table 1A.** FCTR1 (AL031943\_A) nucleotide fragment (SEQ ID NO:1).

accacatctttttctcttcttcgtgctcctaaacttaggctaccaagctttgctggggaaagcactccagg  
tgggtgttactacaaatcaccgctctgctgacccactggtaactacgtacagcctttgatatttcagagt  
caatacctgctttccattctccacagcatcctaataaagtcaggtctctcatctgtcctgcttccccgc  
ttcgcgttcacacgtgtgctgagatatagggaaaggaatgggaacaaggaagcatcgccggcctctcca  
gctctggaggcttcacagcttgccctcctctcgtctgttgagtcacccacagcaaccacaactatgt  
gggagattctgtgcacaggctttggcaact**taa**

The encoded C-terminal fragment of the encoded protein is presented using the one-letter code in Table 1B. The protein including the C-terminal fragment disclosed has a high probability of being secreted extracellularly. A signal peptide most likely is cleaved between residues 19 and 20, *i.e.*, at the dash in the amino acid sequence LLG-KAL.

**Table 1B.** C-terminal fragment of the encoded FCTR1 protein sequence (SEQ ID NO:2).

THLFLFVLLNLGYQALLGKALQVGVTNNHRLTHWYYLTAFDISRVNTCFPFSTASNISHGFSSVLLPR  
FAPTTVLYRERNGNKETAGLSSSGGFTACLLLRLLSHPTNRNHNHYVGDSVPGFGN

In a search of sequence databases, no similarities were found to any known expressed nucleic acid or protein. The human genomic fragment HS223B1, from clone RP1-223B1 on chromosome 6p24.1-25.3, aligned with the FCTR1 nucleotide sequence, as shown in Table 1C.

Putative intron and exon information can be construed from this alignment.

**Table 1C.** BLASTN alignments of FCTR1 (SEQ ID NO:1) with genomic clone HS223B1

Alignment between:

HS223B1 Human DNA sequence from clone RP1-223B1 on chromosome 6p24.1-25.3 Contains STSs and GSSs, complete sequence. 5/2000 and (Pasted\_No.:1-228)

Length = 126281

Score = 452.0, bits (228.0), Expect = 1e-125

Identities = 228/228 (100%)

Strand = Plus / Plus

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Query: 1      acccatctttttctctctctctctctgtctcctaaacttaggctaccagcttctgtggggaaa 60
Sbjct: 1483    acccatctttttctctctctctctctgtctcctaaacttaggctaccagcttctgtggggaaa 1542

Query: 61      gcactccaggtgggtgttactacaaatcacgcgtctgctgacccactggtactacctgaca 120
Sbjct: 1543    gcactccaggtgggtgttactacaaatcacgcgtctgctgacccactggtactacctgaca 1602

Query: 121     gcctttgatattccagagtcataacctgctttccattctccacgacatctaataagt 180
Sbjct: 1603    gcctttgatattccagagtcataacctgctttccattctccacgacatctaataagt 1662

Query: 181     catggcttctcatctgtcctgcttcccgcttcgcttcacactgtg 228
Sbjct: 1663    catggcttctcatctgtcctgcttcccgcttcgcttcacactgtg 1710 (SEQ ID NO:34)

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Alignment between:

HS223B1 Human DNA sequence from clone RP1-223B1 on chromosome 6p24.1-25.3 Contains STSs and GSSs, complete sequence. 5/2000 and (Pasted\_No.:226-381)

Length = 126281

Score = 309.0, bits (156.0), Expect = 5e-82

Identities = 156/156 (100%)

Strand = Plus / Plus

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Query: 226     gtgctgagatatagggaaggaatgggaacaaggaagccatcgccggcctctccagctct 285
Sbjct: 4527    gtgctgagatatagggaaggaatgggaacaaggaagccatcgccggcctctccagctct 4586

Query: 286     ggaggcttcacagcttgccctctctctctgtgttgtagtcatccacacgcaaccacaac 345
Sbjct: 4587    ggaggcttcacagcttgccctctctctctgtgttgtagtcatccacacgcaaccacaac 4646

Query: 346     tatgtgggagattctgtgccaggcttggcacaactaa 381
Sbjct: 4647    tatgtgggagattctgtgccaggcttggcacaactaa 4682 (SEQ ID NO:35)

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The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

#### **FCTR2 (AL078594\_A)**

The novel nucleic acid encoding a novel protein C-terminal fragment is shown in Table 2A. The initiation codon is at the 5' end, and a "TAG" stop codon was identified at the 3' end

indicating that this sequence is a coding sequence. The start and stop codons are shown in bold letters. This sequence originates in chromosome 6, in clone RP1-293L8 at map location q22.2-22.33. Homology of 100% was shown to the human genomic clone HSDJ293L8 obtained from this region, which contains the HEY2 gene for hairy/enhancer-of-split related with YRPW motif 2 (cardiovascular basic helix-loop-helix factor 1, CHF1), ESTs, STSs, GSSs and four putative CpG islands. FCTR2 nucleotide regions 1-213, 214-367, and 366-570 correspond 100% to HSDJ293L8 regions 49502-49714, 52745-52898, and 54432-54636, respectively.

**Table 2A.** Nucleotide sequence (SEQ ID NO:3) of FCTR2 (AL078594\_A).

atgactgtcaaggctcctaaagggtcataaagggtgacataacttctatactgttagttcaaacacttgctc  
agagctgccatgctgtgaggaggcccaagctagt cagctcagagagagcatctggagaggctctgaagct  
acacaactatagagtcctcagctgcacaagccccctgctgttccagctccaaccactgctagactacaac  
catatgatactgagtaacttagccccagagcgtcaggggtgccactgagtatgcagtatgctgacttaataca  
taaaaataaacaccttttagtattcaagcagctcatatcactcacaatttctctttaacaagaaggca  
tgcatcttcatacacggggacaattcggtcagattgtttcttcccaatacctctatgagatcaattgcact  
gaaggaaatgctatttttactagaagaaacgaagggtggaagtcaataatttgaagcatggggtagcttca  
gaggaggagaggttcggggatcgggtacaagacttggtctgggcccaggataaaaatactcagtatgaaaa  
acctgagtag

The encoded FCTR2 polypeptide sequence (SEQ ID NO:4) is presented using the one-letter code in Table 2B. The protein appears not to have a strong probability of secretion. No signal peptide is predicted for this protein. No significant matches were found in a BLASTP search against the FCTR2 polypeptide.

**Table 2B.** Encoded FCTR2 protein sequence (SEQ ID NO:4).

MTVKAPKGGHKGDIITSILLVQTLAQSCHAVRRPKLVSSERASGEALKLHNYRVLSCSPLLFQLQPLLDYN  
HMILSNLAPDVRVPLSMQYADLI IKINTFSIQAAHITHKFLFNKERHAFHTRGQFGQVSSQYLYEINCT  
EGMPITFTRRTKVEVNNFEAWGSRGGEVRSGLRLGLGQDKNTQYEKPE

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

### **FCTR3 (AL078595\_A)**

The novel nucleic acid encoding a novel protein C-terminal fragment is shown in Table 3A. The initiation codon is at the 5' end and a TGA stop codon was identified at the 3' end indicating that this sequence is a coding sequence. The start and stop codons are shown in bold letters. This sequence originates in clone RP3-399J4 on chromosome 6q15-16.3. No significant matches were found in a BLASTN search against the FCTR3 nucleotide sequence.

**Table 3A.** Nucleotide sequence (SEQ ID NO:5) of FCTR3 (AL078595\_A).

atgccgcactgctggctcctgctcttgcctcctgccgccaccacttgacctccccctcttcagccagtggtg  
gtggcagcggctgctcccgacagccaccattcccatcagtaatatggagggggcaaatatgtgtaaagcc

ttcaggtgccaaagctgctccagaacccctggaagaattatcaaagatgctgctcctctcttcaattcca  
 tggatataatcttctccttcagttcttcgcagagcctgcgaatcaaacatgctaaagcagagaaatacaataaga  
 gacctatacttgacattagcagaggaagtcagctgtgtacactaattatgataaacatccattcacaat  
 gtctgggaggagactagccacagagcctggaagaggtgaaagaaacgcacccatgaaaaaggagcaaa  
 tga

The encoded protein is presented using the one-letter code in Table 3B. The protein has a high probability of extracellular secretion. A signal peptide is predicted for this protein with a cleavage site between residues 16 and 17, *i.e.*, at the dash in the amino acid sequence PLA-PPL.

No significant matches were found in a BLASTP search against the FCTR3 polypeptide.

**Table 3B.** Encoded FCTR3 protein sequence (SEQ ID NO:6).

MPPLLVLLLLLPPPLAPLPPLFSQCGSGCSRQPTIPISNMEGQICVKPSGAKAAPEPELELSKMRSLSSIP  
 WYILSFSSAEPAIKHAKAEKYNKRPIIDISRGSPAVVYTNVDKHPFTMSGRRLATDLERGEKHEKHEKGA

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

#### FCTR4 (AL109627\_A)

The novel nucleic acid encoding a novel transforming immortalized mammary oncogene-like protein is shown in Table 4A. An initiation codon is shown at the beginning of the sequence and a TGA stop codon was identified at the 3' end indicating that this sequence is a coding sequence. This sequence originates in chromosome 1 from clone RP4-733M16 at map location p36.11-36.23.

**Table 4A.** Nucleotide sequence (SEQ ID NO:7) of FCTR4 (AL109627\_A).

atggccagacacctccgtgcccggttcggtggttgccccaaactggcagcagagatgccgagggcaaggagt  
 acctggcttgcatctctgcgcagaacccgcgcggggtgtttgggctgcttgagcggccagtgctgctgcc  
 gccctgtgctcatgacactgccagctacaagatcttctgtgccccggaagagtggtgtgggcaagacggcg  
 ctggtggccaaagctggctggcctggaggtgctgtggtgcaccacgagaccacggcatccagaccacgc  
 tggatatttggcagccaaagctgcaggccagcagcgtgtgctcatgttctggttttgagttctgggactg  
 tggagatgtgcactcaaaaagtctgatcatatgctgctggcttgcatggagaacacagatgccttcctc  
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 agggccctgggtgtcgctcaggatgggtcatcggtccaaaattgaccagttacatgcacacggagctgccga  
 gccggacctcacagccttcggcaggctgggagctgccctgctacgggtgaagagtgctgccggggggcg  
 cggctggctgatggggcgacactggacggggcggtggtgcccacagctgcccacatactcaatggcc  
 ttgctgagcagctgtggcaccgagcagacggcgatgacgcccacgacaggacagcagctgtgtcgc  
 gccctgcggcggtcttatgtgcgggactctagggtacatttctgagacgggaaaacctgcatgtgata  
 aaagtgggacagagcgggttcagacgcctcctaactgtccccctgaccccgcatgggttagacttcgtg  
 ctgcctgggaggagaagctgcgaccccgcgccggcgaggagagggcgactccggcagcgcgctggcg  
 cgagaattttcagcggaactggagggggctcttgaactgcctgggtaccaggtaccgggttcagat  
 ctcaactcttgccaatttgctgtaccatactgggcttctgtgggatactggcaagtaaccagccctgg  
 accagcctttggacaaactgagctgcctcttggaccaccaggaaacctgtcttcagatcttcagtc  
 cttctggggcattggccttcttgagcactggaagcaggagtgccaccttggcccccacactgggactgc  
 agtgacttcaggagcaggaggcaatgccagttcagccccccacactgggactgcagcagcttcaggg  
 accaggaggtgatgccaggttcagccctccaccactgggactgcagcgacttcaggagcaggaggagtg  
 cccacatctacagtttctgctccctggccctgcagatgaccagaaacctgacaggttgaaggagccc



tacttccaaccgcacagctgctcttccaccactactcaccagctctgcagccatcctcactgtgctctgtg  
 tgggtgatgattttcttgggtatctgtcataaatttaccatggcatcatcagcattgcaatgttccacactgg  
 caactctgtgctcatgacccaagcgaatgtctcttggggcaatggaggccccaaagcctgagtaaggtg  
 ctctgtgtctgtccaaacaaactgcgggtcctgggtggctgccaactcaggtgcaacagcctcatcatca  
 tcatgtgtggcgcaaacagctgctcaaccacatggaagaatttgttgggctgggaggtggccccgggctga  
 cactccctgctgcccagagctgttgggttctatcaccatctcttgggaagcctctctgctggcacc  
 ctgttctactctgctcaacaaccgggtagagattggactggagcggccacaagtctctgtgcaagtaccagc  
 gaccaatggctggggcggctggacatctggatctgactgctcctctgaggagccatgtgagctgattct  
 gccccggacaatgcgggagctgggtactgggtgaaacgggcaaggccagattctagggagaagg  
 aggggagggaatgcggggttcggagtcgagatccgagagcctctccagaccccgcaaccagatacaagg  
 cctctcgacgtggtgggtggaacctggcctctctactgggaagctgctggctgcatctgcatctggg  
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 ggcagagagagggctctccctgcccgaacagccaaacagcatcagcagcgggctgggcccagagagggc  
 caggtgggtggcagagcaaaagaggaatggactgtgggccaactctgacctccagccccactgactgg  
 gccacctggcactccccaccctgtgacagtggtgccagcaggagatctgctcctttgacagagctgcc  
 gccctgaagcccccgagccagctgtgctcgggacctttccctgtgtccccagaggagctctgggctcctg  
 gcagcgcgtgggtccctggggaccctctgccctctccaagggttgctatggccattatccccaggaggtc  
 agatacacagagatcaccagcgggggatgcggccacagcagggtggcagctgggcacactgtcctgggtgcc  
 cagccccagcctctggaggccactggagtcggcgacacacagcggccggcgccccagcggctg  
 cggagaagaagtctgctggcgcaagatcggggtgtaccagcgtgaagaggtccccgggtgccccagggc  
 ccacgctgcttctcagagcctggcaggtagtgcagagcaggccctgagcacagagagccacgggtg  
 gagtgtctcgggtccaccagcagctcgaaggtcctgagcggaggcctctctcggcatcggagctga  
 tgacccggctgaccttctctgcgcctggggcggaatttcagcagcccgggcactcatctctgggtcagg  
 caccggagccctcgggaagaaagcatctggaatggaggtcgaagtgtcagagatgagcggggaacgg  
 gtgtctcgggccagccccctggtgactcacgagagggcgatgtgtccgagccaggctagacacacaggaag  
 agcgcctttggggctcaggagcaccacagcggcgccagctctcgattctctttaaactccgtctctcta  
 tcaggaataccagcagctggcgagcggcccgaaactgcggcgagcagcggcagggaggggccccgggg  
 gacgaggtcggagggcgagagggggggcgggggcgccggcgccacactctccccacagcagctccttcc  
 gggcgagcgtcggcgaggctccaccttctcgtgtggcaggtatccccgacgtacgcggcgagcgg  
 cgtcctggccacgtgagcctcgggactgcaagctgcaggaggccaagtctgagctgatcacctccgag  
 gcctcctacatccacagcctgtcgggtggctgtggggccacttcttaggtctgcgcgagctgagcaggtgtc  
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 gcaggacctgggacagcggctggaggcagatgtgctgcgtctcagcgtgtgacagctggtgtgaggaccac  
 tgcgggcctctccgagagctacactgccctatgtcaccacacagggctaccaggagcgcacctaccagc  
 gcctgctcctggagaacccagggttccctggcatcctggctgcctggaggagatctcctgtgtgcagcg  
 tctgcccttacctcttcttatcctgccttccagaggatcaccgcctcaagatgttggtggagaac  
 atcctgaagcgggacagcctctgaagacgaagacatggccaccaaaggccttcaatgcgctcaagg  
 agctgtgtgcaggaggtgcaatgcgtagtgtacagtccatgaagagacagaggaaactcatccacctgcagaa  
 gaagatccactttgaggggcaagatttcccgctgatctctcaggccgctggtggttcggcagtgagaga  
 ttggttagagctggcaccactgctgcagcaccctctgccaagctgaagctgtccagcaaggcagctacc  
 tccacctctcaatgactgctgtgctgctctctcggcggaaggagctagggaaagtcttgcggtttctgtcca  
 tgcacagatgctgagctgcagtgaggcagctgagcctgaagctgcaggcctcggggccagcgtgtct  
 ctctcctcagctcctccagggcgagcacatgaagcaccaggtcctgctgcgggccggagcggaaagtgaga  
 agcagcagtgagctcagcctgtgctccctccagccccaggaggacaaggaggtcatcaagtgaaggggga  
 agatgtccccagggttcagtgtgttaggacatcaaggcactgcaccagatgagctgacctgggagaag  
 actgacatcctgtcagtgaggacctggaccagtgacggctggctggaagggttcggcctggcagatgtgtg  
 agaagggtgggtgccccaggcctgtggaagagatcagcagcctcagcggcggctctccgaaacctccg  
 ggaagataagcaggtcacaagtgccaccagcaaaactgggggaggctcctgtgtga

The encoded protein is presented using the one-letter code in Table 4B. The protein has a high probability of sorting into the plasma membrane. No signal peptide is predicted to occur for this protein.

**Table 4B.** Encoded FCTR4 protein sequence (SEQ ID NO:8).

MARPPVPGSVVVPNWHESAEGKEYLACILRKNRRRVFGLLEPVLVPPVSIIDTASYKIFVSGKSGVGKTA  
 LVAKLAGELEVPVHHETGTIQTTVVFWPAKLQASSRVVMFRFEFWDCGESALKKFDHMLLACMENTDAFL

FLFSFTDRASFEDLPGLARLARIAGEAPGVVVRMIGSKFDQYMHMTDVPERDLTAFRQAWELPLLRKSVPGR  
 RLADGRTLDDGRAGLADVAHILNGLAEQLWHQDQTAMTPTPTGRRRLCLAPCGGIYVDPDSRGFTSETGKPALI  
 KVQSGVRELLTVLPTLRVRRLRLRLGGEAATPAAAGERRLRQRWRNENFQRLNEGLFLPGYQVPGSD  
 5 LNSCQLLYPYWACWGWHYKQPLDQPLDKLSCLFDHPGTVFFSIFMSFWGHGLPGALEAGSATLAHHWDC  
 SDFQDQDQAMPPASAPHHWDCSDFDQDQVMPSSALHHWDCSDFDQDQBECHLQFAALALQMTQNPVTGLKEP  
 YFQPHSCLSHLLTSSAAILTVLVCVMMI FLVSVIIYHGIISIAMFHTGNSVLMTQANVLWNGNGGPKALSKV  
 10 LCVCQQQQCGPGGCHIQVTQQLIIIMVGKQLLNHMEEFVGLGGGPGPDTPCLPELQFGFITIFVGAFLAP  
 LFTLLNNRVEILGLDAHFLCKYQRPMAGRGWTSBGSDCSWCPRCELILPRTNARSRLGYWLNQGQILGRR  
 RGGNAGFGVEIREPLQTPQPRYKASRDVGVNLLALFYWKLAVHVLHGFIIAIEGLMNQTLCLGGISPSQL  
 GRERASPAGTAKHQHQRRAWAQRGPGGWSKRGMDCGPPATLQPHLTGPPGTAAHPVAVCQQESLSFAELP  
 15 ALKPPSPVCLDLFFVAPEELRAPGSRWSLGTAPLQGLLWPLSPGGSDBEITSGGMRPSRAGSWPHCPGA  
 QPPALEGFWSPRHTQPORRASHGSEKKSARWKMRYVQREEVPGCEAHAVFLEPGQVQVEQALSTEEPRV  
 ELSGSTRVSLSEGPERRRFSASELMTRLHSSRLRGRNSAARALISGSGTGAAREGKASGMEARSVEMSGDR  
 VSRPAPGDSREGDWSEPRDLTQEEPLGSRSTNERRQSRFLLNSVLVQEYSDVASARELRRQREEGEPG  
 20 DEABGAEEGPGPPRANLSPSSSFRAQRSARGSTFSLWQDIPDVGRSGVLATLSLRDCKLQEAKEFELITSE  
 AYSIHLSVAVGHFLGASSELCLGAQDKQWFLSKLPEVKSTSERFLQDLBQRQLVSGVCDVVLH  
 CPAFRVRYLYPVVNTQAYQERTYQRLLENPRFPGLARLEBSPVCQRLPLTSLFLIPFQRITRLKMLVEN  
 ILKRTAQSGEDEDMAKAFNALKELVQECNASVQSMKRTEELIHLSSKKIHFGKIFPLISQARWLVRHGE  
 25 LVELAPLPAEAPPAKLKLSKAVYLHLFNDCLLSRRKELGKFAVVFVHAKMAELQVRDLSLKQGI PGHV  
 LLQLLHGQHMKHQFLLRARTSESKQRWISALCPSSPQEDKEVISSEGEDCPQVQVCRTYKALHPDELTLK  
 TDILSVRTWTSBGWLEGVRLADGEGWVPQAYVEEISSLSARLNLRENKRVTATSATSKLGEAPV

In a search of sequence databases, it was found, for example, that the nucleic acid  
 sequence has 1120 of 1772 bases (63%) identical to human guanine nucleotide exchange factor  
 25 Rac-GEF cDNA (patn:V99828) (SEQ ID NO: 36), as shown in Table 4C. The terms "percent  
 identities" and "percent positives" are defined below in the Nucleic Acid section.

**Table 4C.** BLASTN identity search of FCTR4 and the hGEF cDNA (SEQ ID NO: 36).

>patn:V99828 Human guanine nucleotide exchange factor Rac-GEF cDNA - Homo sapiens,  
 3171 bp.  
 30 Score = 1856 (278.5 bits), Expect = 7.2e-78, P = 7.2e-78  
 Identities = 1120/1772 (63%), Positives = 1120/1772 (63%), Strand = Plus / Plus

Query: 3042 ATGACCCGGCTGCATCTTCTCTG-CGCTGGGGCGGAATTCAGCAGCCCGGCACT-CA 3099  
 35 Sbjct: 371 ATGAGCCC-CTG-AC-CTTGAATATCCCTGGAGCAGAAATG-CT--CCTTGCAGAAC 423

Query: 3100 TCTCTGG-GTCAGGCACCGAGCAGCCCGGGAAGGAAAGCATCTGGAATGGAGGCTCG- 3157  
 40 Sbjct: 424 GCAATGCAGACGACG-CCAG-G-AGCCGAGAAATGAGTGAAGT-GTCTCCACCCCGGG 479

Query: 3158 AAGTGTAGAGATGAGCGGGGACCGGGTGTGCG-GGCC-A--GCCCTGGTGACTCAC-GA 3212  
 45 Sbjct: 480 AAATGGGCGCAGCCCGAGGAGTGGCGCGCCCTGGCCGACAGCCCAACCGCTACCGA 539

Query: 3213 GAGG--GCGATTGGTCCGAGCCAGGCTAGAC-ACACAGGAAAGGACCGCTTTGGGGGTCC 3269  
 50 Sbjct: 540 GGCCCTGGCGATGATCC-ACCCTATTCGCCGCACTCCTGGAGAAACCTCATTGAACAA 598

Query: 3270 -AGGAGCACCA--ACGAGCGCGGCC-AG-TC--TCGATTCTCTCTT-AACTCGTCTCTCT 3321  
 55 Sbjct: 599 TAGG-GCTCTGTATCAGGAATACCGAGATAAATCGACTC-TCCAAGAAATCGAAAC-C- 654

Query: 3322 ATCAGGAATACAGCGACGTGGCCAGCGCCCGCA-ACTCGCGCGCAGCAGCGCGAG-GA 3379  
 60 Sbjct: 655 AGGAGGCA-ACAG-GATGCAGAAATAGAAGACAATACCAATGGTCCCCGCG-C-AGTGA 710

Query: 3380 GGAGGGCCCGGGGGAGCAGGCGCA-G-GGCCGAG-AGGAGGGGCGGG--GCCGCGCGG 3434  
 65 Sbjct: 711 GGACACCCCGAGGAGGAAGAAAGAGGAGGAGGAGGAGGCGCGCGCCACCA 770

Query: 3435 GCCAACCTCTCCCCAGCAGCTCCTTCGGGGCGCAGCGCTCGGCGCAGGCTCCACCTTC 3494  
 Sbjct: 771 GAGGAAGACTCTGCCC-CAGATC-TGCCGT-CTCAGTAAACC-C-C-A--CTCAAGGTTCT 822

5 Query: 3495 TCGCTGTGGCAGGATATCCCCAGCTACGCGGACGCGCGTCTGTGGCCACGCTG-AGCCT 3553  
 Sbjct: 823 AACCTCTGGCAGGATCTTCCCGAGATCCGAGCAGCGGGTGTGTAGATCCTACAGCCT 882

10 Query: 3554 GGGG-GACTGCAAGCTGCAGGAGGCCAAGTTTGAGCTGATCACCTCCGAGGCCTCCTACA 3612  
 Sbjct: 883 GAGGAGATT--AAGCTGCAGGAGGCCATGTTTCGAGCTGTGTCACTTCCGAGGCGTCTCTACT 940

15 Query: 3613 TCCACAGCCTGTGCTGGCT-GTGGGCCACTTCTTAG-GCTCTGCCGAGCTGAGCGAGTG 3670  
 Sbjct: 941 ACAAGAGTCTGAACCTG-CTGCTGCCACTTCTAGGAGAACGAGCG-GATAGGAAGAT 998

20 Query: 3671 TCTGGGGGCGCAGGACAAGCAGTGGCTGTTTCCAAACTGCCCGAGGTCAAGAGCAC-CA 3729  
 Sbjct: 999 CCTGCACCGTCCGAGGCGCACATCCTCTTCTCCAACTGCTGAGAGTGCTG-GCTGTCA 1057

25 Query: 3730 GCGAGAGTTCTCTGAGGACCTGGAGCAGCGGCTGGAGGAGATG-TGCTGCGCTTCAG- 3787  
 Sbjct: 1058 GTGAGCGGTTGGTTCCTGGAGCTGGAGCACCAGATGAGG-AGAACATGCTCATCT-CTGA 1115

30 Query: 3788 CGTGTGCGAGCTGGTGTGGAACCACTGCCCGGCTTCCGCGA--GTCTACCTGCCCTA 3844  
 Sbjct: 1116 CGTGTGTGAATCGTG-T--ACCGTTATGCGGCGGACCACTTCTCTGTCTACATCACCTA 1172

35 Query: 3845 TGTACCAACACAGGCTACAGGAGCGCACTACCAAGCGCTGCTCTCTGGAGAACCCAG 3904  
 Sbjct: 1173 CGTGACCACTCAGACCTACAGGAGCGGACCTAAGCAGCTGTCTCAGGAGAAAGGC-AG 1231

40 Query: 3905 GTTCCCTGGCA-TCCTGGCTCGCCTGGAGGA-GTCTCCTGTGTGCCAGCGTCTGCCCTT 3962  
 Sbjct: 1232 CTTTCCGGGAGCTGATCGCGCAGCTAGAGCTCGACCCCAA-GTGCAGGGGCTGCCCTTC 1290

45 Query: 3963 ACCTCCTTCTCTTATCTGCGCTTCCAGAGGATCACCGGCTCAAGATGTTGGTGAGAAC 4022  
 Sbjct: 1291 TCTCTCTCTCATCTGCTTCCAGAGGATCACAGGCTCAAGCTGTGGTGACAGAAC 1350

50 Query: 4023 ATCTCTGAAGCGACAGCACAGGGCTCTGAAGACGAAGA-CATGGCCCAAGGCGCTTCAA 4081  
 Sbjct: 1351 ATCTCTGAAGGGTAGAAGAGAGGCTCTGA-GCGGAGTGCACTGCTTGGATGCTCACAA 1409

55 Query: 4082 TGGCTCAAGGAGCTGCTGCGAGGAGTGAATGCTAGTGTACAGTCCA-TGAAGAGGACAG 4140  
 Sbjct: 1410 GGAGCTGGAATGTTGTTGAAGCATGCAACGAGGGCGT-CAGGAAATGAGCCGACAGG 1468

60 Query: 4141 AGGAACCTCATCCACCTG-AGCAAGAGATCCACTTTGAGGGCAAGATTTTCCGCTGATC 4199  
 Sbjct: 1469 AACAGATGATCAGCATTCAG-AAGAAGATGGAGTTCAAGATCAAGTCCGTCGCCATCATC 1527

65 Query: 4200 TCTCAGGCGCGCTGCTGCTGCTGCGCATGAGAGATTG--GTAGA-G-CTGGCACCAGTCC 4255  
 Sbjct: 1528 TCCCATCTCCGCTGGCTGCTGAAGCAGGGTGAGCTGAGCAGATGTACGCGCCCAAGACC 1587

70 Query: 4256 TGCAGCACCCTGCTGCAAGCT-GAAGCTGTCCAGCAAGCAGTCTACTCCACCTCTTCA 4314  
 Sbjct: 1588 TCCCGGACCC--TGAGGACCAAGAAGCTCTTC--CACGAAATT-TACCTCTCTCTGTTCA 1642

Query: 4315 ATGAC-TGCTGTGCTCTCTCGGCGGAGG-AGCTAGGGAAGTTTGGCGTTTGTGT-C 4371  
 Sbjct: 1643 ACCAGCTGCTGG-TGATCTGCGGCGAGATTCCAGG-AGACAAGTACCAGGTATTGTGACTC 1700

Query: 4372 ATGCCAAGATGGCTGAGCTGAG-GTGGGGACCTGAGGCTGAGCTGCAGGGC-ATCCC 4429  
 Sbjct: 1701 A-GCTCCGCGGG--GA-CTGCTGCGTGTGG-AG--GAGC-TGGAGGACCGGGCCAGAGC 1752

Query: 4430 C-GGCCA-CGTGTTCCCTCCAGCTCTCCACGGGCGACATGAA-GC-A--CCAGTT 4483  
 Sbjct: 1753 CTGGCCAACTGTTTCATCTCTGCGGCTGTGGA-GAAC-GCAGATGACCGGGAGGCCACT 1810

0002154476

Query: 4484 CC-TGCTGCGGGCCCGGACGGAAAGTGAGAAGCAGCGATGGATCTCAGCCTTGTGCCCT 4542  
 Sbjet: 1811 ACATGCTAAAGGGCTCCTCTCAGAGTGAGATGAAGCGTGGATGACCTCACTG-GCCCC- 1868

Query: 4543 CCAGCCCCCAGGAGGAC-AAGGAGGT--CAT-CAG-TGAGGGG-GAAG-ATTGCCCCAG 4595  
 Sbjet: 1869 CAA---C-AGGAGGACCAAGTTTGTTCGTTCACATCCCGCTGTCTGAGATGCCCCAG 1923

Query: 4596 GTTCAGTGTGTTAGGACATACAAGGCACTGCACCCAGATGAGCTGACCTTGGAGAAGACT 4655  
 Sbjet: 1924 GTCCAGTGGTGCACCCATACGTGGCTCAGCAGCCAGACGAGCTGACGCTGGAGCTGCC 1983

Query: 4656 GACATCCTGT-CAGTGAGGACCTGGACCAGTGACGGCTGGCTGGAAGGGTCCGCTGGC 4714  
 Sbjet: 1984 GACATCCTCAACATCCTGGACAAG-CTGACGACGGTGGATCTTTGGCGAGCGTCTG-C 2041

Query: 4715 A-GATGTTGAGAAGGGGTGGGTGCCCCAGGCCTATGTG-GAAGAGATCA-GCAGCCTCAG 4771  
 Sbjet: 2042 ACGACCAAGGAGAGAGGTCTGGTT-CCCCAGCTCCATGACTGAGGAGATCTTGAATCCCAAG 2100

Query: 4772 CGCCCGCCTCCGAAACCTCCGGGAGAATAAGCGAGTC-ACAAG 4813  
 Sbjet: 2101 ATCCGTCCTCCAGAA-CCTCAAGGAATGTTTCGTGTCCACAAG 2142

FCTR4 has an even higher homology to a probable guanine nucleotide regulatory protein TIM (SEQ ID NO: 37; SWISSPROT-ACC:Q12774), as shown in Table 4D. The full amino acid sequence of the FCTR4 protein was found to have 276 of 517 residues (53%), identical to, and 355 of 517 residues (68%) positive with, the 519 amino acid residue human probable guanine nucleotide regulatory protein TIM (oncogene TIM, P60 TIM, transforming immortalized mammary oncogene) from ptnr: SWISSPROT-ACC:Q12774. TIM has transforming activities in NIH/3T3 fibroblasts. See, e.g., Chan *et al.*, 1994 *Oncogene* 9: 1057-1063. The 2.3-kb TIM cDNA encodes a predicted protein of 60-kD containing a Dbl-homology (DH) domain. See, e.g., Online Mendelian Inheritance in Man database accession number OMIM 600888. The DH motif is shared by several signal transducing molecules that are implicated as regulators of small GTP-binding proteins. See, OMIM 600888. Therefore, the TIM oncogene is also thought to be involved in the control of cytoskeletal organization through regulation of small GTP-binding proteins. See, e.g., Chan *et al.*, 1994; OMIM 600888.

**Table 4D.** BLASTX identity search of FCTR4 and hTIM protein (SEQ ID NO:37).

>ptnr:SWISSPROT-ACC:Q12774 PROBABLE GUANINE NUCLEOTIDE REGULATORY PROTEIN TIM (ONCOGENE TIM) (P60 TIM) (TRANSFORMING IMMORTALIZED MAMMARY ONCOGENE) - Homo sapiens (Human), 519 aa.  
 Score = 1275 (448.8 bits), Expect = 3.5e-129, P = 3.5e-129  
 Identities = 276/517 (53%), Positives = 355/517 (68%), Frame = +3

Query: 3285 RRQSRFLINS--VLYQREYSDVASARELRRQREBEQPGDEAGAEQGGPPFRANLSPSSS 3458  
 Sbjet: 6 RRCRK-LINSSQLLYQRYSDVVLNKEIQSQRLLESL--SETPGPPSS-PRQPRKALVSSSES 61

Query: 3459 FRAQRSARGSTFSLWODIPDVRSGLVATLSLRDCKLQEAQFELITSEASYIHSLVAVG 3638  
 Sbjet: 62 Y-LQRLSMASGSLQWEIPIVVRNSTVLLSMTHEDQKLQEVKFELIVSEASYLSRLNTAVD 120

Query: 3639 HFLGSARELSCLGAQDKQWLFSKLPEVKSTSERFLQDLEQRLEADVLRFSVCDVVLHDCP 3818  
 Sbjet: 121 HFQLSTSLRATLSNQEHQWLFSRLQDVRDVSATFLSLEENFENNIFSPQVCDVVLHAP 180

Query: 3819 AFRRVYLPVVTNQAYQERTYQRLLENPRFPGLARLEESPVQCRLPLTSLFILPQORIT 3998  
 Sb|jct: 181 DFRRVYLPVVTNQYQERTYQSLMNSNSNFRVLEKLESPPVQCRLSLKSLFILPQORIT 240

Query: 3999 RLKMLVENILKRTAQSGSEEDMATKAFNALKELVQECNASVQSMKRTEELIHLISKIHF 4178  
 Sb|jct: 241 RLKLLQLNILKRTQPSGSEAEATKAHHALEQLLRDCNNNVQSMRTEELIYLSQKIEF 300

Query: 4179 GKIFPLISQARWLVRHGLVELAPLPAAPPAKLKSSKAVYLHLFNDCLLLSRRELKELGKF 4358  
 Sb|jct: 301 CKIFPLISQSRWLKSGELTALFASAPGLRRKLNTRPVHLHLFNDCLLLSRPREGSRF 359

Query: 4359 AVFVHAKMAELQVRDLKSLKQIGPHVFLQLLHG-QHMKGFLLRARTSEKQKRWISAL 4535  
 Sb|jct: 360 LVFDHAPFSSIRGEKCEMKLHGPHKNLFRFLRQNTQGAQAEFLFRTEQSEKLRLWISAL 419

Query: 4536 CPSSPQEDKEVISEGEDCPQVQCVRTYKALHPDELTEKTDILSVRTWTSBGWLEGVRLA 4715  
 Sb|jct: 420 --AMPRELDLL-ECYNSPQVQCLRAYKPRENDELALEKADVVMVTQSSDGLWLEGVRLS 476

Query: 4716 DGEKGWFPQAYVEEISSALRLNRLNRENKRVTSATSKLGE 4835  
 Sb|jct: 477 DGERGWFPVQQVEFISNPEVRAQNLKRAHRVKTAKLQVLE 516

A multiple sequence alignment for AL109627\_A is given in Table 4E, with the FCTR4 protein of the invention being shown on line 2, in a ClustalW analysis comparing the protein of the invention with related protein sequences. Table 4E depicts a ClustalW alignment of the FCTR4 against proteins from a public database. Human oncogene p60 TIM (SEQ ID NO:37; GenBank Acc. No. Q12774) is on line one, FCTR4 (SEQ ID NO:8) is on line two, and an unknown human polypeptide (SEQ ID NO:38; Acc. No. Q99434) is on line three. Based on this alignment, black outlined amino acid residues indicate regions of conserved sequence (*i.e.*, regions that may be required to preserve structural or functional properties); grayed amino acid residues can be mutated to a residue with comparable steric and/or chemical properties without altering protein structure or function (*e.g.* L to V, I, or M); non-highlighted amino acid residues can potentially be mutated to a much broader extent without altering structure or function.

TABLE 4E

TIM_HUMAN	GGPSRRRCRLNLSOLLVQYSDVILNKEIQSQORLES--LSSTTFPSS-EPQEKALVVS
AL109627	STNRRRCRLNLSNMLVQYSDVASARERKQOREBEGPGDABEAESEGPPFAMNSP
Q99434_Human	-----
TIM_HUMAN	SE-SYLRLGMASSSGSLWQELFVVRNETHLSMTHPEOKLQEVKFELTVSBASYLSTAM
AL109627	SSSFRAQSSARSTFSLWQELFVVRNETHLSMTHPEOKLQEVKFELTVSBASYLSTAM
Q99434_Human	-----MPEITSEFSYCHSLD
TIM_HUMAN	AVHFPOLSTSRRLATLSNNOHQWLFSRLQDVVDVATLSDLENFNNHRSFOCDVVV
AL109627	AVHFTCSAPLSECGAGDKQWLFSLPEVVKSTSRFLQLEORLEAVRREUCDVVLF
Q99434_Human	LVSEFQSHLRATYQWQHHLFSLNILDLVGASQRTFDFLEQRHACVIVDESEELLES
TIM_HUMAN	HAFFRRVYLPVVTNQAYQERTYQRLLENPRFPGLARLEESPVQCRLPLTSLFILPQ
AL109627	HCPARRVYLPVVTNQAYQERTYQRLLENPRFPGLARLEESPVQCRLPLTSLFILPQ
Q99434_Human	HNKQYFHEVAVQSNQEVQORTLQALSSNAARRRARERERRRSGSEELLSFILPQ
TIM_HUMAN	RIIRKLKLDQNILKRTQPSGSEAEATKAHHALEQLLRDCNNNVQSMRTEELIHLISKI
AL109627	RITRLKMLQNILKRTQPSGSEEDMATKAFNALKELVQECNASVQSMKRTEELIHLISKI
Q99434_Human	RIIRKLKLDQNILKRTQPSGSEAEATKAHHALEQLLRDCNNNVQSMRTEELIHLISKI

```

TIM_HUMAN      HF-DKIPFLPLISQSEWLVSQGLTALF-FSPSSGLERKKNITRF-VHHLFNDCLLSRPR
AL109627       HF-DKIPFLPLISQSEWLVSQGLTALF-FSPSSGLERKKNITRF-VHHLFNDCLLSRPR
Q99434_Human   DSKVYISIPILISASWLRGEGFLVNE---ETGIFRKRAISFPCYILFNLVHVVEKKK

5  TIM_HUMAN      GGRFVVFDPHAPFSSIRGERFCMKLHCPHK-----NLSRFFRQNSQCAAEFFFR
AL109627       GLGRFAVSVHAKNAELQVRDLSKLOCHES-----PVLGLCLLHC-CHMKCFPLDR
Q99434_Human   SESYVNVQVQVQNNHIOVEKHEDPSGLPFGGNGRSSSVPHQPVYTLRLNSRSGRGLDLS

10 TIM_HUMAN      TETQSKRNIRNISALAMPREELD---LLECYNSPOVOCHRAYPRENDELAEKADVVVVT
AL109627       ARHSESKRMISALCPSSPCEDKEVISGGEDCPQVOVCYATYVALHPDELTEKTDLSVR
Q99434_Human   SDSASDRARNIVALTHSERQWOG---LSSKGLLPQVETITRFAKQADEWTLQCADVVHVL

TIM_HUMAN      QQSDDGLEGVRLISDGRGGWFFVQCVQEFISNPEVTRACNLSAHRVVKIKLOLIVQQA
AL109627       TWSDDGLEGVRLADGKKGWVQVQVVEISLSLRLNLRNENKQVTSATSKGSGAPV
Q99434_Human   CC-EDGWLVEERLDGDTGWFFPEDEARFTISRVAVEGNGVRMEREDRVVDIV-----

```

From these analyses, it is seen that the FCTR4 AL109627\_A nucleic acid and protein are similar to the TIM oncogene. The transforming gene, designated TIM, encoded a predicted protein species of 60 kDa containing a Dbl-Homology (DH) motif. This motif is also present in other growth regulatory molecules including Bcr, Cdc24, Vav, Ras-grf, and Ect2 which have been implicated as regulators of small GTP-binding proteins. NIH3T3 cells transfected with TIM expression plasmid showed altered growth properties in vitro and were tumorigenic when injected into nude mice. The 6.5 kilobasepair (kb) transcript of the TIM gene was found to be expressed mainly in kidney, liver, pancreas, lung, and placenta.

**Table 4F:BLAST alignment of FCTR4**

BLAST alignment file included sequences:

```

Line 2 > gi|11420361|ref|XP_004812.1| Oncogene TIM [Homo sapiens] (SEQ ID NO:39)
Line 3 > gi|4885633|ref|NP_005426.1| Oncogene TIM [Homo sapiens] (SEQ ID NO:40)
Line 4 > gi|9845277|ref|NP_063920.1| neuronal guanine nucleotide exchange
factor [Mus musculus] (SEQ ID NO:41)

```

```

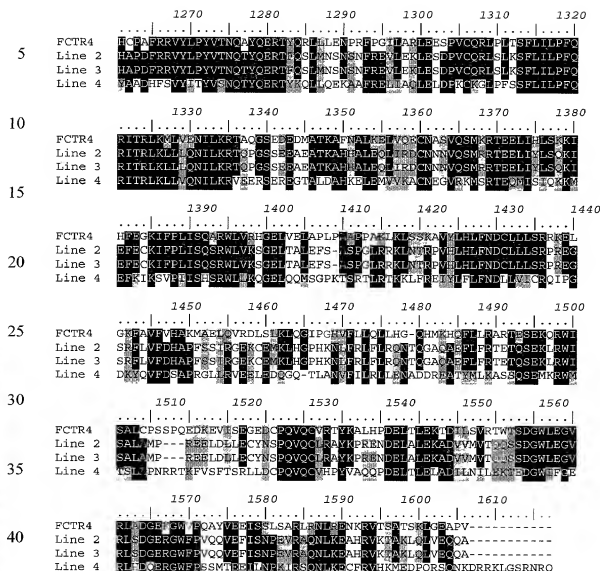
35  FCTR4      1030 1040 1050 1060 1070 1080
Line 2      ALISGSGTGAAREGKASGMARSVEMSGDRVSRFAPCSREGDWSSEPRDTCBEPPLGSR
Line 3      -----EGSSDSRGPAVEKHDFPS-----TVVFEKKPKKEVM
Line 4      -----MIHHPDSWRNLIEQIGLTYCYRDKKSTL

40  FCTR4      1090 1100 1110 1120 1130 1140
Line 2      STNERKCPFLINSVLYQYSDVQASRLNLSQSGEGGSGALAEQGGPFLANLSP
Line 3      GGFRRCCRLINESQLYQYSDVVLNKLQSQORLES---LQPTPCGQ--RPPRQALVS
Line 4      GGFRRCCRLINESQLYQYSDVVLNKLQSQORLES---LQPTPCGQ--RPPRQALVS
Line 4      QEIEH-----RQDQARICNSISQVQCDAGDEEEREEEGDEELASQ---FERRILPQ

45  FCTR4      1150 1160 1170 1180 1190 1200
Line 2      SSSFRACPSARGCTPSLWQDIDVRSQVATLSLRKQLOBAKFRITSEASYVLSNV
Line 3      SE-SYLQRLSMASGSLWCEIPVVENSTHLSNTHEDKLOLVKVELIVSEASYVLSNV
Line 4      SE-SYLQRLSMASGSLWCEIPVVENSTHLSNTHEDKLOLVKVELIVSEASYVLSNV
Line 4      TC---LLSNPHSRFNLWQDIEPLSSQVLDILQPEIRIQAMVELIVSEASYVLSNV
Cons      S L W Q I S V L Q E F H S E A S T S L

55  FCTR4      1210 1220 1230 1240 1250 1260
Line 2      AVGHFLGQASBSECTGATPFWLFSLPKVKSTSERFLQLSQRLAANLVTSQVDDVLD
Line 3      AVDFEQLTSTRATINSQBEQWLFSLQVEDVYSATPLSDLENFNNFISFQVCDVVNL
Line 4      AVDFEQLTSTRATINSQBEQWLFSLQVEDVYSATPLSDLENFNNFISFQVCDVVNL
Line 4      LVSEMERERELKKIKHPSBAHILPSNVLDVMAYSERLLALHHRMENVIVISQVCDVYR

```



FCTR4 was found to have high homology to the domains shown in Table 4G.

**Table 4G: CD domain analysis of FCTR4**

Sequences producing significant alignments:	Score (bits)	E value
Guanine nucleotide exchange factor for Rho/Rac/Cdc42-like GTPa...	110	7e-25
RhoGEF, RhoGEF domain	69.3	1e-12
ras, Ras family	61.2	4e-10
Rab subfamily of small GTPases; Rab GTPases are implicated in ...	51.2	4e-07
SH3, SH3 domain	49.7	1e-06
Rho (Ras homology) subfamily of Ras-like small GTPases; Member...	44.7	4e-05
Src homology 3 domains; Src homology 3 (SH3) domains bind to t...	43.9	6e-05
Ras subfamily of RAS small GTPases; Similar in fold and functi...	38.9	0.002
arf, ADP-ribosylation factor family	38.1	0.003

The AL109627\_A nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in various cancers, tumors and similar neoplastic diseases.

For example, a cDNA encoding the transforming immortalized mammary oncogene-like protein

may be useful in gene therapy, and the transforming immortalized mammary oncogene-like protein may be useful when administered to a subject in need thereof. The novel nucleic acid encoding transforming immortalized mammary oncogene-like protein, and the transforming immortalized mammary oncogene-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These materials are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods.

### **FCTR5 (AL109913\_A)**

The novel FCTR5 nucleic acid encoding a C-terminal fragment of a novel FCTR5 protein is shown in Table 5A. This sequence contains no initiation codon. A TAG stop codon was identified at the 3' end indicating that this sequence is a coding sequence. The stop codon is shown in bold letters. This sequence originates in chromosome X, clone RP11-183K14, and is found at map location q26.3-27.3.

**Table 5A.** FCTR5 (AL109913\_A) C-terminal nucleotide fragment (SEQ ID NO:9).

```

natgatgatgagcaaaacatgatttcaatattgagcctgggtgctgtgacacattgctgtgttcacccag
ttgctgtgacagtcacatgatcaacaagtctgcacacatgacctctcatctccatattccagtgcacaggt
attcctttcccaactgcaggccccccaacaggatgtgggcagcctgcatctccgtggactggagccaa
aatgccaaagcagcagcactcttcagttccatgcctccagaaggcctgtccctgcgcactgggatggtgc
ttgtttgcaagggtatagatgagaaaactgctgccttgcggaaggaaagggtgctgtttggtctcttcgc
tggcatcccatcttttaggaattccagcccaacaagcgcctccaattag

```

The encoded C-terminal fragment of the encoded protein is presented using the one-letter code in Table 5B. The C-terminal fragment disclosed has a very high probability of being secreted extracellularly. A signal peptide most likely is cleaved between residues 28 and 29, i.e., at the dash in the amino acid sequence CDS-HDQ.

**Table 5B.** Encoded FCTR5 polypeptide sequence (SEQ ID NO: 10).

```

XDDEQNMSILSLVSVTIIVFIPVACDSHDQQVCTMTFSSPYVPVKLFLSPTAGPPTGCGQPASPLDWSQ
NAKAQHLRVPCLQKGLSLRTGMVLVCKVIDEKTAALSEGKVLFGLFAGIPIFRNSSPNKPPSN

```

In a search of sequence databases, no similarities were found to any currently disclosed nucleic acid or protein.

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.



**FCTR6 (AL109928\_A)**

A novel nucleic acid encoding a novel transmembrane protein is shown in Table 6A. It was identified in chromosome 20 clone RP4-551D2 at map location q13.2-13.33. An initiation codon is shown at the beginning of the sequence and a TAG stop codon was identified at the 3' end indicating that this sequence is a coding sequence. These are shown in bold face in Table 6A.

**Table 6A. Nucleotide sequence (SEQ ID NO:11) of FCTR6 (AL109928\_A).**

atgagatccgggaggcaccctcgctgctgctgcttctagtctgctgctgtggctgctgcaggtcagta  
tcatctgacagctgtccaacaggaacacagatgatcttactaagcaacaaggtgctactataagttccagga  
aaagatctaccagctcttcacggcgatccaagagaagatgggttatcaccaccttggagctggaggaggaa  
gaccocgggaccccttcccaaacctcatttggtgagctgttcaataatatgtcttataacatgtcactaatgt  
atctaactcagtggaactggtgtggatgaatatccagagattgggttgtttctctagaagatcatgagaa  
cggaaggtatataatgttcacgcgcctgtcgatcgagaaatgacaccatcttcacgagctggacagcaagg  
gtgcctctccctcagggtcttcgcggggatgagcagaggccatctacgggaagggttgggtgctgggttatt  
ttgatgttggggagcgtccaacaggaaaattgtggatacatcttgcatttcaacattaggatcagtgga  
tgtgaatgatcatgcagcccgagttccagagagaaggaatttaacatcactgtgcagaaaaaaccaatctgca  
gggcaacctatttttcagatggttagcagtcgatctggatgaagaaaacacatccaaattctcgaagtccttt  
acttccctcatttctcaaacaccattactgaaagaaagtgggttccgggttgatgccttagtggagaaat  
acgactctctggctgcttagattatgagaccgctcctcagtttaccactgctaatacagagccagggactgt  
ggagaaccgctcactgtcatccagcaccacgcttcacgtggatgtgcaagaaggcaacaaccacagggcctg  
catttaccaggagaactataaggttcagattcctgaaggccgagcagcaggcggtgttgctgctcct  
ggttcaagatcgagattctccatttaccatcagcttggagagcaaaattcaacatttgatggtgcaatgaa  
gaggggcattttgacatttcgactgacctgagaccaacgaagggtatataaatgttatcaagcctttgg  
attatgagactcgcgcagcgcgaagcctcatcattgtcgtggagaatgaggagagctcgtctctctgtga  
gagaggaagagcttcagcgcgcgaagggaagcagcagccagccactgtgagtgtcgaggtgacagagcc  
aacgacccacccagcctttacccccagagcttcaattgtcaataaagagaggggcgcagcgcctgggaccc  
tgttgggaacttttaatgcctgatccagacagccagataagatatgaactggttcagaccagcaaa  
ttgggtcagcgtcgacaaaaactccggagtggtcatcacctggagccaattgaccgagaatcccccat  
gtaataaacagtttttatgtaatcatcattcagctgttgatgatggcttccccacgcagactgctacag  
ggaccctaattgctctcctgtgtgacatcaatgacaacgctcccgaactctccgcacgcttcccgctacat  
ggaggctctgtgagctctgctgtgcatgagccctccacatcgaggcagaggatccggacctggagccgctt  
tctgaccactttatcatttgaattggacaataacctggggaaaatcgggaggacacacaggaagtggggagaa  
attggggaactctcctcatcagggggtaggaggctgctgggagtcctcgagacataattctgcacatcgg  
caagaagggtgttccagggaagctccaggattgacgtcaactgtttggcctgggtcaatcagttgaactt  
ttaaccttgagaagcctgccagctgggttaattacttgggtgcactctcattggagacaaacagggaactt  
ccagaaagcaaacctgtccatgtaaggatctgccccctgtgccagtgggctcacaatgtgtggagcttgcaga  
tgcagaagtggggcttcatgtgggggcccgtgtccctgtctgtgcagctatttggctctggcagctggct  
ctgctttttctgttgcgatgctatttttgtgcttgaacctaaaggagtcggatgctctgataccaatgatg  
aaggccaccaaacactggtcattgtataatcgggagagcaaaaggcaacttcagcccgagacatggtcagatgt  
tgaaggccagagggcgggtctcgtcatctgcacagctcgacagggacccagcaggaggttaaggggagg  
gaacccaaagcctccacctctcaggttttgggtgatctctgggttccctcagtgctcctgcacaaatattgtta  
gatctcgaggaagtgctcctcatctgcagcagtcagtcagcccaagcagctgtgctctggggagctgga  
tagcacagagaccagatccacagacatgggcccagatgagcaggagactgcagcagcccatcatgggaa  
acaattggcagccctgcagaatgggtgctgctggcactgcttcaagacaacacagacatctctccgg  
gctcagaagccttgctcaaaagcagggaagccaggctcctcgagaagggggctgtgtaccacagcatca  
gggctgcaggggccttccccaggtcctgactgtgaaactggaaatggggctggaggacagagaaagaaaca  
gaggctcttggggaggcttcatggccaggctggctgcccagctgaagggggactatctgcagagcttgg  
gaaggggagctccacagtggaactcctgtgttgaaggagccagatccctcacactggcaggccaaaaa  
ggcctggatccccaaactttacaaaaagagaaataaatcaacaacagttagcactatagtcaacaacgtga  
gcatactgtcaacaacatgacacctatagtcaacaacgttagcactatagtcaacaacgttagcactat  
tagtcaacaacgttagcactatagtcaacaacgttagcactatagtcaacaacatagcactatagtcaa  
caacgttagcatctatagtcaacaatgcacttcaacattttactttaaagtgttaggatacatgtgcagaa

gtgcagctctaaagagagaaatcgcttcagcctcagcaggggctgcatcatccccagggaagagccacag  
 ctggggcaggattgccacaagacattacaaggagatgatgccaggagactaacgcagactggtaaacg  
 gaaacacggggctttggctcgaacacccctctttcaagaaagtgtttatgaccacaaggaaagtctctctc  
 atctgtgtgggtcaacaactccccaagaatccccccggcacaacatccctggatcagaacccatcagtggt  
 5 tccctatgctcctgggaatttccattcaatggcctccgaacatgagcctgctcttctgctgaagccca  
 aaacccagatcagatctttaccccaagagacatcttgggctccctccaggctcttctgctactctgtg  
 ccctcatctcggagtcctgtccccacccctatctacagaaactccacagccctcctggctgccccgatg  
 gtctctcgacaggagactgtctacctcccgaggtcagctgtgggtctggtcctcttgccatcatggc  
 10 agagattttgctgattctccccctggctggtgctctgctacactcctccagagtgtttaacaagag  
 ctgaggatgctgagctgccagggacttggtgtaggtggcagatg

The encoded protein is presented using the one-letter code in Table 6B. The protein has a high probability of sorting into the plasma membrane. Cleavage of a signal peptide is predicted to occur between residues 27 and 28, i.e., at the dash in the sequence IDS-VQQ.

**Table 6B.** Encoded FCTR6 protein sequence (SEQ ID NO:12).

MRSGRHPSLLLLLLVLLQVSIIDS VQQETDDLTKQTKCHYKFQEKIYQPLRRSKRRVITLLELEE  
 DPGFPFKLIGELFNNMSYNNMMLYISGPGVDEYPEIGLFSLEDHENGRIYVHRPDRREMTSPFTSWTAR  
 VPSSRASAGMSRGHLREGLVLVYFDVVERSTGKIVDTSLIFNIRISDVNDHAPQFPKEFNITVQENQSA  
 20 GQPTFQMLAVLDLEENTPNSQVLYFLISQTPLLKESGFRVDRLSGEIRLSGCLDYETAPOQFTLLIRARD  
 GEPSLSSTTTVHVDVQEGNNHRPAFTQENYKVQIPEGRASQGVRLRLVQDRDSPFTSAWRKFNILHGNE  
 EGHFDISTDPETNEGLNVIKPLDYETRAQSLIIVVENEERLVFCERGLQPPRKAASATVSVQVTD  
 NDPPAFHGPSFIVNKEEGARPTLLGTTFNAMDPDSQIRYELVHDPAFNWVSDKNSGVVITVEIDRESPH  
 VNNSEFVYIIHAVDDGFPPTATGTLMLFLSDINDNVPTLRPSRYMEVCESAVHEPLHIEAEDPDLEPF  
 25 SDPFTFELDLNMTWGAEDTWKLGRLGNSPHQVGCGWESLRHLILASGKKGVSRAPGLTSLFGLGQSVEL  
 LTLRLSLPRGNLYVPLFIDGKQGLSQKQTVHVRICPCASGLTCVELADAEGVLHVGLFVPCAFAVALAVA  
 LLFLLCRYFVLEPKRHGCSVSNDEGHQTLVMYNAESKGTSAQTWSDEVGQRPALICTAAAGTPTQGVKGR  
 EPKPPSPSRFWCISGFPVSCKYCRSRGSASTICSESVSPSTLCSGELSDSTQIHRHGPDBEQETASSPSWE  
 TMGSPAEWLPGTCFTKTQTSPLGELPKSRQARLLQKGAUVYPTQGCRLPOVLTAELMEGLEDRERT  
 30 EALGEAFMARLAADLKG DYLSLGRFASTVESCVRSQSPSHWQAKAWIPKLLQKRKNFNNVAPIVNNV  
 ASIVNNIAPIVNNVAPIVNNVASIVNNVAPIVNNVAPIVNNVAPIVNNVAPIVNNVAPIVNNVAPIVNNV  
 VQSKERNRFLSRGCIIPQGRATAGRGLPQDIYKEMMPRLTQTGKRKHGALARTPSFKKVVYDHKEVSL  
 ICWVQTSPEDPPIPIWIRTHQWFPSEAWFFPFGNLRMTSLPFLPEAQNPVRSRLPQDRPWSASLQAFAYS  
 35 PSSWSVPPTPIYRNSTSPPGCPDGPRTGRLVYLPRSRVSGSGLAIMAEIILYPLAAGALLTSSRVVKN  
 LRLMSCPGTWLQVA

In a search of sequence databases, it was found, for example, that the nucleic acid sequence has 225 of 381 bases (59%) identical to human cadherin-13 coding sequence (patn: T85405) (Table 6C).

**Table 6C.** BLASTN identity search of FCTR6 and hCAD-13 (SEQ ID NO:42).

>patn:T85405 Human cadherin-13 coding sequence - Homo sapiens, 2690 bp.  
 Score = 323 (48.5 bits), Expect = 6.4e-05, P = 6.4e-05  
 Identities = 225/381 (59%), Positives = 225/381 (59%), Strand = Plus / Plus

Query: 804 TCCTCAGTTTACACTGCTAATCAGAG-C-CAGGGACTG--TGGA--GAACGCTC-ACTGT 856  
 45 Sbjct: 1416 TCCCAAGTATGAACATGATCATC-GAGGCTCAAGATGAGCTGACTGATGTGGATTAA 1474

Query: 857 CATCCACGACCAACCGTTACAGTGGATGTGCAA-GAAGGCAACACACAGGCGCTGATTT 915  
 50 Sbjct: 1475 CAGGACGCGCCACAGC-CACGATCATGATCGATGACAAAAATGATCATTACCAAAATTC 1533

Query: 916 ACCCAGGAGAACTATAAGTTCAGATTCTCTGAAGGCCGAGCCAGCCAGGCGGTG-TTG-C 973  
 Sbjct: 1534 ACCAAGAAAGATTTCAGAC-CACAGTCGAGGAAG--GAGCT-GT--GGGAGTTATTGTC 1587

15

20

25

55

60

```
Query:   157 LRSKRKRVVITTLLEEEDPGFFPKLIGELFNNSYNMSLMYLISGPGVDYPEIGLFSL 336
      ||+||| ||| + | |||| + + ++ |+|| ++|| | + | |+ +
Sbjct:   154 LQKKRDKVTPPINLPENSRRFPQSELVRISRDRDNLSLRYSVTGPFGAQ- PPTGFIII 212

Query:   337 EDHENGRIYVHRPVDREMTSPSF 402
      ++ + | + + || | 
Sbjct:   213 NPI-SGQLSVTKPLDREOIARF 233
```

>Homo sapiens cadherin-like protein VR20 mRNA, partial cds (GenBank AF169690)

20

		1810	1820	1830	1840	1850	1860
FCTR6	hvr20	AGACATATTCTTGCATCTGGCAAGAAGGTTTTCACAGGAAGCTCCAGGATTGAOCTCA					
5							
		1870	1880	1890	1900	1910	1920
FCTR6	hvr20	CTGTTTGGGCTGGGTCATCAGTGAACCTTTAACTTGAGAAGCCTGCCACGTGTAAT					
10							
		1930	1940	1950	1960	1970	1980
FCTR6	hvr20	TACTTGTGTCACCTCTTCATTGGAGACAAACAGGACTTTCCAGAAAGCAACTGTCCAT					
15							
		1990	2000	2010	2020	2030	2040
FCTR6	hvr20	GTAAAGGATCTGCCCTGTGCCAGTGGGCTCACATGTGTGGAGCTTGACAGATGCAGAAGTG					
20							
		2050	2060	2070	2080	2090	2100
FCTR6	hvr20	GGGCTTCATGTGGGGGCCCTGTTCCCTGTCTGTGCAGCAATTGTGGCTTGCCAGTGGCT					
25							
		2110	2120	2130	2140	2150	2160
FCTR6	hvr20	CTGCTTTTCTGTGCGATGCTATTGTGTCTGAACCTAAGAGGCATGGATGCTCTGTA					
30							
		2170	2180	2190	2200	2210	2220
FCTR6	hvr20	TCCAAATGATGAAGGCCACCAACACTGGTTCATGTATTAATCGCGAGAGCAAAAGGCACCTTC					
35							
		2230	2240	2250	2260	2270	2280
FCTR6	hvr20	GCCAGACATGTCAGATGTTGAAGGCCAGAGGCCGGCTCTGCTCATCTGCACAGCTGCT					
40							
		2290	2300	2310	2320	2330	2340
FCTR6	hvr20	SCAGGACCCACGCGAGGAGTTAAGCTTTTCCAGCTGTCACATGTCACAGACAACTC					
45							

Table 6G shows a ClustalW alignment of FCTR6 with related proteins found in public databases. FCTR6 polypeptide is on line 5, human CAD2 (SEQ ID NO:46) is on line 1, bovine CAD2 (SEQ ID NO:47) is on line 2, mouse CAD2 (SEQ ID NO:48) is on line 3, and chicken CAD2 (SEQ ID NO:49) is on line 4. Based on this alignment, black outlined amino acid residues indicate regions of conserved sequence (i.e., regions that may be required to preserve structural or functional properties); grayed amino acid residues can be mutated to a residue with comparable steric and/or chemical properties without altering protein structure or function (e.g. L to V, I, or M); non-highlighted amino acid residues can potentially be mutated to a much broader extent without altering structure or function.

**Table 6G.** ClustalW alignment including FCTR6 (AL109928\_A) protein.

CAD2_HUMAN	NCRIAGALRTHLP-----LALALQASVSESGEIALCKTGFPEDVVSVAISKDVHEGQPL
CAD2_BOVIN	-----SLCKTGFPEDVVSVAISDVHEGQPL
CAD2_MOUSE	NCRIAGGRGTHLP-----LALALQASVSESGEIALCKTGFPEDVVSVAISKDVHEGQPL
CAD2_CHICK	NCRIAGTPPPRLPLPLALMLALQAPIKRKEEDMLCKTGFPEDVVSVAISVHGGQPL
AL109928_genscan2	MSGSG-----RHS-----SLLLLLVLLVLLVSTHESVQQETD

CAD2\_HUMAN LNVKFSNCNGKRKVQYESSEPADFKVDEDCGVYAVRSFPLSSSE-HAKFLIVAQDKETQEK  
 CAD2\_BOVIN LNVKFSNCNGKRKVQYESSEPADFKVDEDCGVYAVRSFPLSSSE-HSKFLIYAQDKETQEK  
 CAD2\_MOUSE LNVKFSNCNGKRKVQYESSEPADFKVDEDCGVYAVRSFPLSSSE-QMKFLIYAQDKETQEK  
 CAD2\_CHICK LNVKFSNCNGKRKVQYESSEPADFKVDEDCGVYAVRSFPLSSSE-PTESFVMSMDKETQEBE  
 AL109928\_genscan\_2 DLNLTQKCH--YDFEKKIQE--LRRSKR--RNVITLLESEEDQPPSEFKLIGLFLNNMS

CAD2\_HUMAN AQVAVKLSSKEILTESVKESEAEVEEIVPPKRCFSKHSCHQKQKRDWVIPPINLPENSRC  
 CAD2\_BOVIN AQVAVKLSSKEILTESVKESEAEVEEIVPPKRCFSKHSCHQKQKRDWVIPPINLPENSRC  
 CAD2\_MOUSE AQVAVKLSSKEILTESVKESEAEVEEIVPPKRCFSKHSCHQKQKRDWVIPPINLPENSRC  
 CAD2\_CHICK AQVAVKLSSKEILTESVKESEAEVEEIVPPKRCFSKHSCHQKQKRDWVIPPINLPENSRC  
 AL109928\_genscan\_2 YNMSMYLLIS---GPGVDFYFETG--EFSLEDHENG-----RNVHRRVDR--EMFSP

CAD2\_HUMAN PFPQELVIRISDRDKNLSLRYSVTGPGADQPTTGIFIIINPISGQLSVTKPLDRELIARFH  
 CAD2\_BOVIN PFPQELVIRISDRDKNLSLRYSVTGPGADQPTTGIFIIINPISGQLSVTKPLDRELIARFH  
 CAD2\_MOUSE PFPQELVIRISDRDKNLSLRYSVTGPGADQPTTGIFIIINPISGQLSVTKPLDRELIARFH  
 CAD2\_CHICK PFPQELVIRISDRDKNLSLRYSVTGPGADQPTTGIFIIINPISGQLSVTKPLDRELIARFH  
 AL109928\_genscan\_2 FTISWIDVPSSE-----AS--CMSSR-----STL-----RSGLVLYVFD

CAD2\_HUMAN LRAHAVDINGNQENPIDIVINVIDMNDNRPEFLHQVWNGTVPEGSKPGTYVMTVTAIDA  
 CAD2\_BOVIN LRAHAVDINGNQENPIDIVINVIDMNDNRPEFLHQVWNGTVPEGSKPGTYVMTVTAIDA  
 CAD2\_MOUSE LRAHAVDINGNQENPIDIVINVIDMNDNRPEFLHQVWNGTVPEGSKPGTYVMTVTAIDA  
 CAD2\_CHICK LRAHAVDINGNQENPIDIVINVIDMNDNRPEFLHQVWNGTVPEGSKPGTYVMTVTAIDA  
 AL109928\_genscan\_2 TVKRST---KRVITSLIFNHRKSDNCHNPFQPEKSENNIVQNGSKAQPTFOMLRDEL

CAD2\_HUMAN DDPNALNGMLRYRILSQAPSTPSNNMFTINNNTGDIITVAAGLDREKVVQOYTLIIQATDN  
 CAD2\_BOVIN DDPNALNGMLRYRILSQAPSTPSNNMFTINNNTGDIITVAAGLDREKVVQOYTLIIQATDN  
 CAD2\_MOUSE DDPNALNGMLRYRILSQAPSTPSNNMFTINNNTGDIITVAAGLDREKVVQOYTLIIQATDN  
 CAD2\_CHICK DDPNALNGMLRYRILSQAPSTPSNNMFTINNNTGDIITVAAGLDREKVVQOYTLIIQATDN  
 AL109928\_genscan\_2 ESBTHSQVLPNPLSCSTLILK-ESCEPMDRLSCBIR-LSGCHLYETAPQPTVILKAPDC

CAD2\_HUMAN EGNPTVGLSNTATAVITVTDWNDNPEETAMTFYGEVGPENRVDVIVANLTVTDKQDPHTE  
 CAD2\_BOVIN EGNPTVGLSNTATAVITVTDWNDNPEETAMTFYGEVGPENRVDVIVANLTVTDKQDPHTE  
 CAD2\_MOUSE EGNPTVGLSNTATAVITVTDWNDNPEETAMTFYGEVGPENRVDVIVANLTVTDKQDPHTE  
 CAD2\_CHICK EGNPTVGLSNTATAVITVTDWNDNPEETAMTFYGEVGPENRVDVIVANLTVTDKQDPHTE  
 AL109928\_genscan\_2 ESBG--LSPVTVIHHVQGGNHR--PPT--ENKRVQVGPENRVSQGLLIL--QGLD--SPHPS

CAD2\_HUMAN LANAIRISGGDPTGRFATLDPNSNDGLVTVVKPIDFETNRMFVLTVAAENG---VPLA  
 CAD2\_BOVIN LANAIRISGGDPTGRFATLDPNSNDGLVTVVKPIDFETNRMFVLTVAAENG---VPLA  
 CAD2\_MOUSE LANAIRISGGDPTGRFATLDPNSNDGLVTVVKPIDFETNRMFVLTVAAENG---VPLA  
 CAD2\_CHICK LANAIRISGGDPTGRFATLDPNSNDGLVTVVKPIDFETNRMFVLTVAAENG---VPLA  
 AL109928\_genscan\_2 LKRLKNNILFNEE--LH--LSDPTDSE--LNN--TKL--ETPRAGS--LTVNNEERLAFCE

CAD2\_HUMAN KGQIHPQ---STATVSVTVIDVNEPNFYAENPKIIROEGGLHAGCLMTTAAQDPDRYM  
 CAD2\_BOVIN KGQIHPQ---STATVSVTVIDVNEPNFYAENPKIIROEGGLHAGCLMTTAAQDPDRYM  
 CAD2\_MOUSE KGQIHPQ---STATVSVTVIDVNEPNFYAENPKIIROEGGLHAGCLMTTAAQDPDRYM  
 CAD2\_CHICK KGQIHPQ---STATVSVTVIDVNEPNFYAENPKIIROEGGLHAGCLMTTAAQDPDRYM  
 AL109928\_genscan\_2 RKLQPRKAAK---TVSKVTLV--LNPENPESVTVNKEG--PFTALLSTF--LDPG--

CAD2\_HUMAN QQM--IRYTKLSDPANWLKIDPVNGQITTVAVLDRESYVYNNIYNATPLASDNGIPPMSC  
 CAD2\_BOVIN QQM--IRYTKLSDPANWLKIDPVNGQITTVAVLDRESYVYNNIYNATPLASDNGIPPMSC  
 CAD2\_MOUSE QQM--IRYTKLSDPANWLKIDPVNGQITTVAVLDRESYVYNNIYNATPLASDNGIPPMSC  
 CAD2\_CHICK QQM--IRYTKLSDPANWLKIDPVNGQITTVAVLDRESYVYNNIYNATPLASDNGIPPMSC  
 AL109928\_genscan\_2 -SQ--IRVELVHDPANWTVKNSGVTVIYEFPTDRSSVYVYNNIYNATPLASDNGIPPMSC

CAD2\_HUMAN TGTPLQIYLLDINDNAPQVLPQE---ETCETPPINSINIATLDYIDPNAGFAFALR---LK  
 CAD2\_BOVIN TGTPLQIYLLDINDNAPQVLPQE---ETCETPPINSINIATLDYIDPNAGFAFALR---LK  
 CAD2\_MOUSE TGTPLQIYLLDINDNAPQVLPQE---ETCETPPINSINIATLDYIDPNAGFAFALR---LK  
 CAD2\_CHICK TGTPLQIYLLDINDNAPQVLPQE---ETCETPPINSINIATLDYIDPNAGFAFALR---LK  
 AL109928\_genscan\_2 TGTPLMFLSDINDNAPQVLPQESRYM--CESAVHSP--HIS--EPPHLEFSLTE--LSTLNTW

CAD2\_HUMAN ---LSPVTIKRNNI-----LIRNGDPAQLN-----LK  
 CAD2\_BOVIN ---LSPVTIKRNNI-----LIRNGDPAQLN-----LK  
 CAD2\_MOUSE ---LSPVTIKRNNI-----LIRNGDPAQLN-----LK  
 CAD2\_CHICK ---LSPVTIKRNNI-----LIRNGDPAQLN-----LK  
 AL109928\_genscan\_2 GNABDTWIKRNNI--NSPHQGVGGCWESLRHILASGKIGV--BEAFOLTS--FGLQGVRL--L

000221.16434760

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CAD2_HUMAN      IKFLEAGIYEVPIIITDSGNPPKSNISILRVKVCQDCSNGDCTI--VDRIVGAGLGTGA--
CAD2_BOVIN      IKFLEAGIYEVPIIITDSGNPPKSNISILRVKVCQDCSNGDCTI--VDRIVGAGLGTGA--
CAD2_MOUSE      IKFLEAGIYEVPIIITDSGNPPKSNISILRVKVCQDCSNGDCTI--VDRIVGAGLGTGA--
CAD2_CHICK      IKFLEAGIYEVPIIITDSGNPPKSNISILRVKVCQDCSNGDCTI--VDRIVGAGLGTGA--
5  AL109928_genscan_2  ERSPPHGNYVYVTFPRK--QGLESKQTMHVNITCGASGLTVELADARVGLHYALFPV

CAD2_HUMAN      --IAILLCTIILLILVLMFVVMKRR--DKERQAKQLIDPED--DV--
CAD2_BOVIN      --IAILLCTIILLILVLMFVVMKRR--DKERQAKQLIDPED--DV--
CAD2_MOUSE      --IAILLCTIILLILVLMFVVMKRR--DKERQAKQLIDPED--DV--
10 CAD2_CHICK      --IAILLCTIILLILVLMFVVMKRR--DKERQAKQLIDPED--DV--
AL109928_genscan_2  CAAPVAIAWALLETFRCYFVLEPKRHGCSVSNDEGHETLVMYNAESKGTSAQTWSDEGQ

CAD2_HUMAN      RDNLKYDEEGG--GEEDQD--YDLS--QLQPPDTPVEPDAIKPVG
CAD2_BOVIN      RDNLKYDEEGG--GEEDQD--YDLS--QLQPPDTPVEPDAIKPVG
CAD2_MOUSE      RDNLKYDEEGG--GEEDQD--YDLS--QLQPPDTPVEPDAIKPVG
15 CAD2_CHICK      RDNLKYDEEGG--GEEDQD--YDLS--QLQPPDTPVEPDAIKPVG
AL109928_genscan_2  RPAHITCTAARPTQGVKREBKPPPSRFNCTSGFPSVSCYKCRSGSASICSESVSEST

CAD2_HUMAN      IRR--MD--ERPIHAE--PQYPVRSAAHPFGDIG--DFINEGLKAANDNPTAF--PV
CAD2_BOVIN      IRR--MD--ERPIHAE--PQYPVRSAAHPFGDIG--DFINEGLKAANDNPTAF--PV
CAD2_MOUSE      IRR--MD--ERPIHAE--PQYPVRSAAHPFGDIG--DFINEGLKAANDNPTAF--PV
20 CAD2_CHICK      IRR--MD--ERPIHAE--PQYPVRSAAHPFGDIG--DFINEGLKAANDNPTAF--PV
AL109928_genscan_2  ICSGSDSTETQIRHGHDEQETASSSEWETGSPAEHWLPTCTCFKTQTSSGLEALPK

CAD2_HUMAN      DSLLYFDYEGSGS--TAGSLSS--LNSSSSGGGEQDYDYLNDWGPRFKKLADMYGGGDD
CAD2_BOVIN      DSLLYFDYEGSGS--TAGSLSS--LNSSSSGGGEQDYDYLNDWGPRFKKLADMYGGGDD
CAD2_MOUSE      DSLLYFDYEGSGS--TAGSLSS--LNSSSSGGGEQDYDYLNDWGPRFKKLADMYGGGDD
25 CAD2_CHICK      DSLLYFDYEGSGS--TAGSLSS--LNSSSSGGGEQDYDYLNDWGPRFKKLADMYGGGDD
AL109928_genscan_2  SROARLLQKFAVYPOKTCRRLPQVITAELEMLDPRERTAEIGERPMKLAADLTQYIL

CAD2_HUMAN      -----
CAD2_BOVIN      -----
CAD2_MOUSE      -----
30 CAD2_CHICK      -----
AL109928_genscan_2  QSLGREASTVESCVRSGSQSPSHWQAKKAWPKLQKKNKFNVPVIVNVASIVNNTAPI

CAD2_HUMAN      -----
CAD2_BOVIN      -----
CAD2_MOUSE      -----
35 CAD2_CHICK      -----
AL109928_genscan_2  VNNVAPIVNNVASIVNNVAPIVNNVAPIVNNVAPIVNNVASIVNNALGHFTLSARIHVQK

CAD2_HUMAN      -----
CAD2_BOVIN      -----
CAD2_MOUSE      -----
40 CAD2_CHICK      -----
AL109928_genscan_2  VQSKEKRNFRSLRGCIIPQGRATAGRLPQDIYKEMMPRLTQTGRRKHGALARTPFSFKK

CAD2_HUMAN      -----
CAD2_BOVIN      -----
CAD2_MOUSE      -----
45 CAD2_CHICK      -----
AL109928_genscan_2  VVVDHKEVSLICWQTSPPDPPPHIPWIRTHQWFPASAWFFPNGLRMTSLPGLPRAQNPVS

CAD2_HUMAN      -----
CAD2_BOVIN      -----
CAD2_MOUSE      -----
50 CAD2_CHICK      -----
AL109928_genscan_2  YRSLPQRPSWASLQAFAYSVPSSWSVPPTPIRNSNTPPGCPDGRTGRVLVYLPSPRSVGS

CAD2_HUMAN      -----
CAD2_BOVIN      -----
CAD2_MOUSE      -----
55 CAD2_CHICK      -----
AL109928_genscan_2  GPLATMAEILLYPLAAGALLTSSRVVNKEIRMLSCPGTWLQVA

CAD2_HUMAN      -----
CAD2_BOVIN      -----
CAD2_MOUSE      -----
60 CAD2_CHICK      -----
AL109928_genscan_2  -----
CAD2_HUMAN      -----
CAD2_BOVIN      -----
CAD2_MOUSE      -----
65 CAD2_CHICK      -----
AL109928_genscan_2  -----

```

From these analyses, it is seen that the FCTR6 AL109928\_A nucleic acid and protein a weak resemblance to neural cadherin, and a strong resemblance across a portion of FCTR6 with

human cadherin-like VR20. Cadherins are calcium dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types. N-cadherin may be involved in neuronal recognition mechanism. They are type I membrane proteins.

Finally, FCTR6 was found to have high homology to the domains shown in Table 6H.

**Table 6H:** CD domain analysis of FCTR4

Sequences producing significant alignments:	Score (bits)	E value
cadherin, Cadherin domain	73.9	5e-14
cadherin, Cadherin domain	57.0	6e-09
cadherin, Cadherin domain	44.3	4e-05
cadherin, Cadherin domain	40.4	6e-04
Cadherin repeats.; Cadherins are glycoproteins involved in Ca2...	56.6	8e-09
Cadherin repeats.; Cadherins are glycoproteins involved in Ca2...	49.3	1e-06

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

#### **FCTR7 (AL109953\_A)**

The novel FCTR7 nucleic acid encoding a novel secreted FCTR7 protein is shown in Table 7A1. This sequence contains an initiation codon at the 5' end, and a TGA stop codon was identified at the 3' end indicating that this sequence is a coding sequence. An alternative novel FCTR7A nucleic acid encoding a novel secreted protein is shown in Table 7A2. This sequence contains an initiation codon at the 5' end, a frameshift at position 61, and a TAA stop codon indicating that this sequence is a coding sequence. The start and stop codons for both sequences are shown in bold letters. These sequences originate in chromosome 20 clone RP4-746H2.

**Table 7A1.** FCTR7 (AL109953\_A) nucleotide sequence (SEQ ID NO:13).

atgggatgcagactgctgacccctgctgtgtttccctacaacctgcttccagctcctcgtggctctttggct  
cccaatccagagctttcggaacaccagagccctgtgcctctccctgcagctggctgggagttccaggg  
cattaacacagacagctcttggccatcagccagtgactgtatggagcttggatgtgaatacacagctcct  
gcacccctccgaggcatctccacaccgtctcccagagaaatgtctcgtaaaagctgctcctcttggggagg  
ctctgggctttggagagagcacctggaaattcccactagaaaagcccaaaaactga

**Table 7A2.** Alternative FCTR7A (AL109953\_A) nucleotide sequence (SEQ ID NO:29).

atgggatgcagactgctgacccctgctgtgtttccctacaacctgcttccagctcctcgtggctctttggctc  
ccaatccagagctttcggaacaccagagccctgtgcctctccctgcagctggctgggagttccagggc  
atcaacacagacagctcttggccatcagccagtgactgtatggagcttggatgtgaatacacagctcctg  
cattccctccgaggcatctccacaccgtctcccagagaaatgtctcgtaaaagctgctcctcttggggaggc  
tctgggctttggagagagcacctggaattcccactagaaaagcccaaaaactga



The encoded FCTR7 protein is presented using the one-letter code in Table 7B1. The FCTR7 protein has a low probability of being secreted extracellularly, although a signal peptide most likely is cleaved between residues 17 and 18, *i.e.* at the dash in the sequence ASS-SSW. The encoded FCTR7A protein is presented using the one-letter code in Table 7B2.

**Table 7B.** FCTR7 protein sequence (SEQ ID NO:14) encoded by SEQ ID NO:13.

MGCRLTLTLLCFLQPASSSSWLFSGSRAFANTRAPVPLPAAGWEFGQINTDSLCPASDCMELGCEYTAP  
ASLRGISTPSPRECLVKAAPLGEALGFGEWNSPLEKPKN

**Table 7B.** FCTR7A protein sequence (SEQ ID NO:30) encoded by SEQ ID NO:29.

MGCRLTLTLLCFLQPASSSSWLSLAPNPELSRTPEPLCLSLQLAGSSRALTQTVFAHQPVTVWSLDVNTQLL  
HPSEASPHRLPENVS

In a search of sequence databases, no similarities were found to known nucleic acid or protein.

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

### FCTR8 (AL110115\_A)

The novel nucleic acid encoding a novel secreted protein is shown in Table 8A. This sequence contains an initiation codon at the 5' end, and a TAG stop codon was identified at the 3' end indicating that this sequence is a coding sequence. This sequence originates in chromosome 20 clone RP3-324O17.

**Table 8A.** FCTR8 (AL110115\_A) nucleotide sequence (SEQ ID NO:15).

atgaagctcctctctctgtcttttgactgttactctgtctcctggcccagggtcaccccagggtctgccagcca  
tgaaactctctttacctgttctcttgccatcctcttgcccatagaagaaccagtgatatacagtagagtgttg  
gatggatggacactgcgggtgtgtgtgcaaaagatggtgaagacagcatcatacgtgccgaaatcgtaaa  
cgggtgtgtgttctcagtcgttatttaacaatccaaacagtaacaattcatggaatccttggtggacca  
ctcctcagatgtgccacaacagctccaaaaatgaagacaaatataactaatagatag

The encoded protein is presented using the one-letter code in Table 8B. The protein has a moderate probability of sorting to the plasma membrane. A signal peptide most likely is cleaved between residues 43 and 44, *i.e.* at the dash in the sequence VIS-VEC.

**Table 8B.** Encoded FCTR8 protein sequence (SEQ ID NO:16).

MKLLLLLLTVTLLLAQVTPGLPAMKLLYLFLAIIAIEEPVISVECWMDGHCRLLCKDGEDSIIRCRNRK  
RCCVPSRYLTIQVVTIHGILGWTPQMSTTAPKMKTNITNR

In a search of sequence databases, the BLASTN comparison revealed 91 of 129 bases (70%), out of a total of 413 bases, are identical to an unidentified human secreted protein. No similarities of significance were identified at the amino acid level.



3' end indicating that this sequence is a coding sequence. The start and stop codons are shown in bold type. This sequence originates in chromosome 20 clone RP4-770C23.

**Table 10A.** FCTR10 (AL118509 A) nucleotide sequence (SEQ ID NO:19)

**a**tgcaactcactgcggttctcactgcttttgggttgctgttctctgtgcactgctatccttctcttccc  
 ctacgtaggggtttctggaactggcgccagctgtgctcactcagacaggtaggggctctctaatatggtt  
 ctatgatccacaatcagatctaaaaaacaacaaaaataaaaacacacaaaaaagagataggaaggaag  
 gaggcgcctctgtacagctctaaacatctgaaatccactgggtagcagctctgtcagaactagctccaggga  
 aggagagaggttcagccagcttaggaacagctatcattacagaagggaatttaggaaaaagcaagaaggt  
 gatgaagaatgagaggggaggaagaaaaagggaaaagggaacaactccagcttctcacaatctcctct  
 gaaagacgtacactgcccattggcgaggcagctctgattatgggttagtaaacccaactctgaaatccaaa  
 atccaaaattctacaacatcccaaatctttgagtgcgaactgtgatgatcaatggaagtgtctac**ta**g

The encoded protein is presented using the one-letter code in Table 10B. The protein has a high probability of being secreted extracellularly. A signal peptide most likely is cleaved between residues 27 and 28, *i.e.* at the dash in the sequence TVG-FLD.

**Table 10B.** Encoded FCTR10 protein sequence (SEO ID NO:20).

MHSLRFLLLLWLLFPLSLLSFSSPTVGFLDCGTVVTSDQVRALLIMFYESQSDLKTNKNKTKQKQKREGK  
ERSVNVNWKWSTGDQPLSELSSRKEEVQPVEEPVSLSEGNLGKSKKVMKNEREEKKEKEQTSSFSQFPS  
ERRTLPARHAGYGLSNPNLKIONPKCYNIPNVLSANVMINGNVH

In a search of sequence databases, the BLASTN comparison (see Table 10C) revealed 90 of 117 bases (76%), in a large genomic fragment originating on chromosome 6q23.1-24.3, are identical to a human DNA sequence containing the MEKK5 (ASK1, MAPKKK5) gene for MAP/ERK kinase kinase 5 (Mitogen Activated Protein kinase kinase kinase 5), as well as ESTs, GSSs and a putative CpG island. No similarities of significance were identified at the amino acid level.

**Table 10C.** BLASTN of FCTR10 with MEKK5.

>gb:GENBANK-ID:HS325F22|acc:AL024508 Human DNA sequence from clone 325F22 on chromosome 6p23.1-24.3. Contains the MEKK5 (ASK1, MAPKKK5) gene for MAP/ERK kinase kinase 5 (Mitogen Activated Protein kinase kinase kinase 5), ESTs, GSSs and a putative CpG island, complete sequence - Homo sapiens, 154788 bp.

Score = 330 (49.5 bits), Expect = 1.9e-10, Sum P(2) = 1.9e-10

Identities = 90/117 (76%), Positives = 90/117 (76%), Strand = Plus / Plus

Query: 444 AAGGCACGCTGGATATGG--GTTAAGTAACCCCAATCTGAAAATCCAAAATCCAAAATGC 501

Sbjct: 145356 AAGGCACACTGTAATAACAAGTTGAGTAACCCCTAATAAAAAAATCTGAAATCTAAATATGC 145415

Query: 502 TACAACATCCCAAATGTTTTGAGTGCCAATGTGATGATCAATGGAAATGTTCACTAG 558

Sbjct: 145416 TCCAAATCCAAACTTTTGTAGTGCCAAACATGATGCTCAAAGGAAATGCTCATTGG 145472 (SEQ ID NO:51)

Score = 163 (24.5 bits), Expect = 1.9e-10, Sum P(2) = 1.9e-10  
Identities = 69/96 (71%), Positives = 69/96 (71%), Strand = Plus / Plus

Query: 145 GAATCACAATCAGATTTAAAAACAAACAAAATAAAA-CAAAA-CAAAAACAAAAAAGAG 202

Sbjct: 29978 GAGTGAGACTCCG-TCTCAAAA-AAACAAAAACAAAAACAAAAACAAAAACAG 30035

Query: 203 AAGGGAAGGAGCGGTCTGTGAACGTTAACAAATGGAAA 240

Sbjct: 30036 AA--ATGCATCCATAT-T-AAC-TTC-CAAAATGCAAA 30066 (SEQ ID NO:52)

5 Score = 121 (18.2 bits), Expect = 1.4e-08, Sum P(2) = 1.4e-08  
 Identities = 57/86 (66%), Positives = 57/86 (66%), Strand = Plus / Plus

Query: 94 GGCACAGTTGTCACTTCAGACCAGGT-A-AGGGCTCTATT-AATTAIGTTCATGAATCA 150  
 Sbjct: 13513 GGCACATTTT-ACFTT-GAGTGATTACATTGCTTTACTCAAAGAAGTGGTGGAAATGG 13570

Query: 151 CAATCAGATTTTAAAAACAAACAAAAATAA 179  
 Sbjct: 13571 CTAA-AGTTTAAAAACAAACAAAACTAA 13598 (SEQ ID NO:53)

15 Score = 117 (17.6 bits), Expect = 2.0e-08, Sum P(2) = 2.0e-08  
 Identities = 27/30 (90%), Positives = 27/30 (90%), Strand = Plus / Plus

20 Query: 171 CAAAAATAAAACAAACAAAAACAAAAAG 200  
 Sbjct: 101741 CAAAAATAAAACAAACAAAAAG-AATAAAG 101769 (SEQ ID NO:54)

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

### FCTR11 (AL118522\_A\_EXT)

The novel nucleic acid encoding a novel K<sup>+</sup> channel-like protein is shown in Table 11A. This sequence contains an initiation codon at the 5' end, and a TAA stop codon was identified near the 3' end indicating that this sequence is a coding sequence. The start and stop codons are shown in bold type and a putative 3'UTR is underlined. This sequence originates in chromosome 20 and was assembled as a consensus extension using the 8 sequences FCTR11 AL118522\_genscan\_2+, est:gb\_AA283204+, est:gb\_AI091631-, est:gb\_AI097455+, est:gb\_AI690321+, est:gb\_AI739096+, est:gb\_AI968607+, and est:gb\_AW073155+.

**Table 11A.** FCTR11 (AL118522\_A\_EXT) nucleotide sequence (SEQ ID NO:21).

**ATGCGGAGGCCGAGCGTGC**CGCGCGCGGGCTGGTCTGTGCACCCCTGTGTTACCTGCTGGTGGGGCGCTGCTGCTTCTGACGCGCTCGAGTCCGAGGCGGAAGCGGCCGACGCGACTGCTGGTCCAGAAAGCGGGCGCCTCTCCGGAGGAAGTTCTGGGCTTCTCGGCCGAGGACTACCGCGAGCTGGAGCGCCTGGCGCTCCAGGCTGAGCCCCACCGCGCGCGCCAGTGGAAAGTTCGCCCGCTCCTTCTACTTTCGCGCATCACCGTCATCACTACCATCGAGTACGGCCACGCGCGCGCGGGTACGGACTCCGGCAAGGTCCTTGCATGTTCTACGCGCTCTGGGCATCCCGCTGACGCTGGTCACTTTTCCAGAGCCTGGGCGAACGCGGTGGTGGCGGCCCTCTGTGGCGCCAAAGTGTGCTGCTGGGCGCTGCGGTGGACGTGCGTGTCCACGGAGAACCTGGTGGTGGCCGGCGTGCTGGCGTGTGCCGCCACCTGGCCCTCGGGGCCGTGCGCTTCTCGCATTCGAGGGCTGGACCTTCTCCACGCTACTACTGCTTCATCACCTCACCACCATCGGCTTCGGCGCACTTCGTGGCACTGCAGAGCGGAGCGCTGCAGAGGAAGTCCCTACGTGCGCTTCAGCTTCTCTACATCCTCTGGGGCTCACGGTCTATTGGCGCTTCTCAACACCTGGTGGTCTCTGCGCTTCTCTGTCGCGAGCGCGACTGGCCCGAGCGCGCTGCCCGCACCCCGAGCGCGCCCCCGGGGGCGCCGAGAGCCGTGGCCCTCTGGGCTGCCCGCGCCCGGCCGCTCCGTGGGGTCTCGCGCTTCTGTCTTCTGCCACGTGCACAAGCTGGAGAGGTCGCGCCGCGACAACCTGGGCTTTTGCGCCCTCGAGCCCGGGGGTCTGCGTGGCGGGCAGGCTCCAGGCTTGGGGCCGGTGGAAGTCCATCTGACAACCCACCCAGGCCAGGCTCGAATCTGGAAATGGGAGGCTCTGGCTTCACTATCAGGCACCCCTCCCGAGGATTGGAACCGGATGACGGGCTTTAGGCGGTTTTTGTCCACGAGCAGTTTTTTC

The encoded protein is presented using the one-letter code in Table 11B. The protein has a high probability of being sorted to the plasma membrane. A signal peptide most likely is cleaved between residues 23 and 24, *i.e.* at the dash in the sequence VGA-AVF.

MRRPSVRAAGLVLCCLCYLLVGAAGVFDALAESEASGRQLLVQKRGALRRKFGFSAEDYRELERLALQAE  
PHRAGKQWPGPGSFYFAITVITIEYBHAAGPTDSKGVCFMCFYALLGILPLTLTVQSLGERLNAVRRLL  
LAAKCLGLRWTVCSTENLVVAGLACAATLALGAVASFHEGWTFHHAYICYFTLLTIGDFDFAVRLS  
GEALQRKLPYVAFSFLYIILLGLTVIGAFNLVVLRLFLVASADWPERAARTPSRPPGAPESRGLWLP  
RPSVGSASFVCHVKLERCARDNLGFSPPSPSGVPPGQQAFLRGARWKSIXQHPGQGRILWNGRVWLQLS  
HGGPQGLETDGGLGGFLGPRAFVHYCLWLSPLPPGQITVTP

**Table 11C.** BLASTN of FCTR11 with TWIK (SEO ID NO:55).

Shift: 479 C C C G G C T C A C G C T C G T C A T G T T C C A G A G C C T C G C C G A G C C G A T C A A C A C C T T G C C T C A C C G T 520

30

35

40

45

50

55

60

Line 3  
 250 260 270 280 290 300  
 5 FCTR11  
 Line 2  
 Line 3  
 310 320 330 340 350 360  
 10 FCTR11  
 Line 2  
 Line 3  
 370 380 390 400 410 420  
 15 FCTR11  
 Line 2  
 Line 3  
 430 440 450 460 470 480  
 20 FCTR11  
 Line 2  
 Line 3  
 490 500 510 520 530 540  
 25 FCTR11  
 Line 2  
 Line 3  
 550 560 570 580 590 600  
 35 FCTR11  
 Line 2  
 Line 3  
 610 620 630 640 650 660  
 40 FCTR11  
 Line 2  
 Line 3  
 670 680 690 700 710 720  
 45 FCTR11  
 Line 2  
 Line 3  
 730 740 750 760 770 780  
 50 FCTR11  
 Line 2  
 Line 3  
 790 800 810 820 830 840  
 60 FCTR11  
 Line 2  
 Line 3  
 850 860 870 880 890 900  
 65 FCTR11  
 Line 2  
 Line 3  
 910 920 930 940 950 960  
 70 FCTR11

00221-164000

Line 2	CCGCGCCCGCTCCGCTGGGCTCGGCTCTGCTTCTGCCACGTGCACAAGCTGGAGAGGTG
Line 3	CCGCGCCCGCTCCGCTGGGCTCGGCTCTGCTTCTGCCACGTGCACAAGCTGGAGAGGTG
5	970 980 990 1000 1010 1020
FCTR11	.....
Line 2	CGCGCCGCAACAACCTGGGCTTTTCGCCCCCTCGAGCCCGGGGTCTGTCGTGGCGGGCT
Line 3	CGCGCCGCAACAACCTGGGCTTTTCGCCCCCTCGAGCCCGGGGTCTGTCGTGGCGGGCT
10	1030 1040 1050 1060 1070 1080
FCTR11	.....
Line 2	GGCTCCAGGCTTGGGGCCCGGTGGAAGTCCATCTGACAACCCACCAGGCAGGGTCTG
Line 3	GGCTCCAGGCTTGGGGCCCGGTGGAAGTCCATCTGACAACCCACCAGGCAGGGTCTG
15	1090 1100 1110 1120 1130 1140
FCTR11	.....
Line 2	LVCTCGGAATGGGAGGGTCTGGCTTCAGCTATCAGGGCACCTCCCCAGGGATTGGAAC
Line 3	LVCTCGGAATGGGAGGGTCTGGCTTCAGCTATCAGGGCACCTCCCCAGGGATTGGAAC
20	1150 1160 1170 1180 1190 1200
FCTR11	.....
Line 2	GGATGACGGGCTTAGGGCGGTCTTTCGCGCGAGCTTTTTCATTACTGTCTGTGGC
Line 3	GGATGACGGGCTTAGGGCGGTCTTTCGCGCGAGCTTTTTCATTACTGTCTGTGGC
25	1210 1220 1230 1240 1250 1260
FCTR11	.....
Line 2	TAAAGCCCTCCCTCTTTCCAAAAATATATTACAGTCAACCCATATGCCCGGAGGAA
Line 3	TAAAGCCCTCCCTCTTTCCAAAAATATATTACAGTCAACCCATATGCCCGGAGGAA
30	1270 1280
FCTR11	.....
Line 2	AAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO:56)
Line 3	AAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO:57)

A BlastP search against the FCTR11 protein also identified FCTR11 as having high homology to the potassium channel proteins TASK and KT3.3, as shown in Table 11E. Line 1 shows the FCTR11 polypeptide (SEQ ID NO:22), line 2 is the human TASK protein (gi|10944275|emb|CAC14068.1| (AL118522) dJ781B1.1 (A novel protein similar to the acid sensitive potassium channel protein TASK (KCNK3)) [Homo sapiens])(SEQ ID NO:58), line 3 is the human KT3.3 protein (gi|11228686|gb|AAG33127.1|AF257081\_1 (AF257081) two pore potassium channel KT3.3 [Homo sapiens]) (SEQ ID NO:59), and line 4 is the guinea pig TASK3 protein (gi|7546839|gb|AAF63706.1|AF212827\_1 (AF212827) potassium channel TASK3 [Cavia porcellus])(SEQ ID NO:60).

**Table 11E: BlastP search of FCTR11 protein**

10 20 30 40 50 60	.....
FCTR11	MRPSPVRAAGLVLTCLCYLLVGAAVFDALSEAESESGRORLLVQKRGALRRKFGFSAEDYR
Line 2	MRPSPVRAAGLVLTCLCYLLVGAAVFDALSEAESESGRORLLVQKRGALRRKFGFSAEDYR
Line 3	MRPSPVRAAGLVLTCLCYLLVGAAVFDALSEAESESGRORLLVQKRGALRRKFGFSAEDYR
Line 4	MRPSPVRAAGLVLTCLCYLLVGAAVFDALSEAESESGRORLLVQKRGALRRKFGFSAEDYR
55	70 80 90 100 110 120
FCTR11	BLERLALQAEPRHAGRWKFPFGSPYFAITVITLIEVGHAAFGTDSKGVFCGFYALLGIFL



```

          370          380          390
      .....|.....|.....|.....
FCTR11  DGPLGGFLPRAVFHYCLWLSPLPPQKYITVTP
Line 2                                     (SEQ ID NO:58)
Line 3  ----- (SEQ ID NO:59)
Line 4  DNHRLMLRRKSV----- (SEQ ID NO:60)

```

>ptnr:SPTREMBL-ACC:O35111 CTBAK - MUS MUSCULUS (MOUSE), 409 aa.  
Score = 832 (292.9 bits), Expect = 1.0e-85, Sum P(2) = 1.0e-85  
Identities = 168/258 (65%), Positives = 200/258 (77%), Frame = +1

Query: 718 NLVVLRLVASADWPERAA 774  
 |||||++|+|+|  
 Sbjct: 240 NLVVLRFMTMNAEDEKRD 258 (SEQ ID NO:61)

Query: 941 SCVAGRLPGLPGGSPSDNPTQAR---VESGMGGSGF 1042  
 ||++||| | | | | | | | | | | + | | | |

Sbjct: 277 SCLSG---SLGDGVRPRDPVTCAAAAGVGVGVGSGSF 311 (SEQ ID NO:62)

Score = 40 (14.1 bits), Expect = 1.9e-84, Sum P(2) = 1.9e-84  
Identities = 13/39 (33%), Positives = 16/39 (41%), Frame = +2

Query: 941 SCVAGRLPGLPGGSPSDNPTQARVESGMGGSGFSYQGT 1057

+|| | | | | + + | | | |  
Sbjct: 353 TCVEHSHSSPGGGGRYSPTPSHPCLCSGTQQRSAISSVST 391 (SEQ ID NO:63)

Potassium channels are ubiquitous multisubunit membrane proteins that regulate membrane potential in numerous cell types. One family of mammalian K<sup>+</sup> channels is characterized by the presence of 4 transmembrane domains and 2 P domains per subunit; this family includes TASK, TWIK (KCNK1; OMIM 601745) and TREK (KCNK2; OMIM 603219). See, Duprat *et al.*, 1997 *EMBO J.* 16: 5464-5471. The human cDNA, designated TASK, encodes a 394-amino acid polypeptide with 85% identity to the mouse ortholog. See, Duprat *et al.*, 1997. The sequence contains consensus sites for N-linked glycosylation and for phosphorylation at the C-terminal. Northern blot analysis showed that TASK is expressed in a variety of human tissues, with highest levels in pancreas and placenta. See, Duprat *et al.*, 1997. Expression of the TASK cDNA revealed that the functional protein creates currents that are K<sup>(+)</sup>-selective, instantaneous, and noninactivating. See, OMIM 603220. These currents showed an outward rectification when external K<sup>+</sup> was low, but evinced absence of activation and inactivation kinetics as well as voltage independence, characteristics of so-called leak or background conductances. See, OMIM 603220. TASK currents were very sensitive to small changes in extracellular pH, suggesting that TASK has a role in cellular responses to changes in extracellular pH. See, OMIM 603220.

Finally, FCTR11 was found to have high homology to the domains shown in Table 11G.

**Table 11G: CD domain analysis of FCTR11**

Sequences producing significant alignments:	Score (bits)	E value
TWIK_channel, TASK K <sup>+</sup> channel	284	5e-78
CNG_membrane, Transmembrane region cyclic Nucleotide Gated Cha...	35.8	0.004

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

### **FCTR12 (AL121574\_A)**

The novel nucleic acid encoding a novel protein C-terminal fragment is shown in Table 12A. A TAG stop codon was identified at the 3' end indicating that this sequence is a coding sequence. This sequence originates in clone RP3-441A12 of chromosome 6.

**Table 12A.** FCTR12 (AL121574\_A) nucleotide sequence (SEQ ID NO:23).

natcagactctattgaccgcactctaacgttgtagcagcattgtgtggaattgtgtccttgtggccttggg  
 catttaagcttctactacttgacctctatagttttggcactcttctatacacatgactatcagcaagctaa  
 attatttactgactgtcctgctccccgcactccgctttagggcgcggaacgaagtggcagcccgatc  
 ccagctgatcagcggctgggcttggcgcttgctccccggcgagacacattgtgactcctcgggagggg  
 cgcacgcggggagggggcgagcggccattgtccggtcagcgcagcctccgggggaggggacgggtgta  
 cggagacagcagggcccggggcttcagagcggcgctgcgactccggagcggcgggggctccggtcct  
 tcctcgcgccacgcacaggacatctctctggtcgggagcggcggtgagaccgccgagggcgctgtg  
 tcctcctcccccggtcctcgagcggggcccgggcccgccgcccagcgtgccgcgcgagctc  
 cgcgcgcgcgagcaccatgggagacgctgggagcgcgagcgaacgcgccagcctgcgcctcgctg  
 tcctcgcggtctctggggactaacgcaggttcctttaggatgtctgctcttccgagtgactaggctgca  
 ggacttgctgccagcattgccagtcaggacactaatcagtggtcggttgaa**tag**

The encoded C-terminal fragment of the encoded protein is presented using the one-letter  
 code in Table 12B. The C-terminal fragment disclosed has a very high probability of being  
 sorted to the plasma membrane. No cleavage site for a signal peptide was detected.

**Table 12B.** Encoded FCTR12 protein sequence (SEQ ID NO:24).

XQTLTATLTLGSIIVSLWLWAFKLHYLTSIVLASSTHSDYQAKLFDTCPAPRTPLLRGTQKHARI  
 PADQLRGLFGVSGPGRDHCDSSGGAHAGEGAERPLSGQRSLRGRGRCYGDSTRARGFRAAAATPEPAGGSGP  
 SLRHRTHGLSGWGAARPAEGVCVPPPPRSSSGARAQPPPLPPSSAAAHHRRRWERAQQAQPAASL  
 SLRLLTNGSSFRIAALSSDLGCRCTCCPALPSQDTNQCGSVE

In a search of sequence databases, no similarities were found to any known nucleic acid  
 or protein.

The nucleic acids and proteins of the invention are potentially useful in the treatment of  
 cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune  
 disorders, and hematopoietic disorders.

**FCTR13 (AL121723\_A)**

A novel nucleic acid encoding a novel secreted morphogenic protein is shown in Table  
 13A. It was identified in chromosome 20 clone RP5-854E16. An initiation codon is shown at  
 the beginning of the sequence and a TGA stop codon was identified at the 3' end indicating that  
 this sequence is a coding sequence. These are shown in bold face in Figure 13A.

**Table 13A.** Nucleotide sequence (SEQ ID NO:25) of FCTR13 (AL121723\_A).

**atg**cgccatccgctgggtcctgctgctgctcctctctgcccctggtagacctcctcactgcagcctctatcc  
 acgattctcatgcccaagagagctccttgggtcttacaggcctccagagcctactccaaggcttcagccg  
 acttttctcgtacgatcctgcttcggggcatagacagctcttctctgccccatggacttcggggc  
 ctccctaggaaactaccaacaagaggagaaacaggaggacaccagctgaggaaacaacccctctccagccacc  
 tccatattgacaaggtgaccgacaataagacaggagaggtgctgatctccgagaaggtgggtggatccat  
 ccagccggcgagggggagcttcgagggttaactggaaggcggcgccctgggtccatccggaaggctatg  
 gacaaacttccatgcagagctccatccccgggtggcctttaggatcatgaagctgccacgggtggagggtccc  
 accacaatgctcctggaggcgccgctggctcagtgagaaagcagacaccgctgcaggccatcaggatgg  
 gctccacagggggaccgcgaggacgtcctaaaagaggggaccagggtcctccactccgggctgtccc  
 tcgaaagaccacttactgtacatcttcaggcttctcctggcagctataggggttgggacggggagcac  
 ctgcaagctgggttggtgtcctgggtcagcgtatcaaggcctggcagctatggacacagggttgggctt  
 ggagctcggtaccagtggtgagacttctggaatgcgttcaggaggctacagtaaccaaacaatcatg

gtactagtacaaaaacggatatacatagaccaatgcaacagaaacagagagccagaaataagccacacacc  
tacaaccatctgatatcttcgacaaagctga

The encoded protein is presented using the one-letter code in Table 13B. The protein has  
a very high probability of secreted extracellularly. Cleavage of a signal peptide is predicted to  
occur between residues 28 and 29, *i.e.* at the dash in the sequence AHA-QES.

**Table 13B.** Encoded FCTR13 protein sequence (SEQ ID NO:26).

MRHPLVLLLLLSALVTSFTAASIHDAHAQESSLGLTGLQSLQGFSRLFLLKDDLLRGIDSFSPAMDFRG  
LPRNYQQEENEHQLRNTLTSSHLHIDKVIDNKTGEVLISEKVVASIQFAEGSFEGNWKAAALVSIKAM  
DNFHAELHPRVAFWIMKLPRWRSHHNVLGGRWLSEKRLHQLAIQDGLHEGTREDVLKEGTQSSSHSGLS  
SERPTYCTSSGFPGSYRGWDRGAPASWVGWVSVSKGLAHGPTGLGLEPSSGIDFVNAFMEGYSNQNM  
VLVQKRIHRPMQONREARNKATHLPSPDLRQS

In a search of sequence databases, it was found, for example, that the nucleic acid  
sequence has 356 of 388 bases (91%) identical to human cysteine-rich secreted protein-like-N  
cDNA (patn: : V07910) (Table 13C). The full amino acid sequence of the protein was found to  
have 166 of 218 residues (76%), identical to, and 181 of 218 residues (83%) positive with,  
human dickkopf-1 (dkk-1) having a total of 242 amino acid residues. This protein (soggy-1  
protein) is a member of a novel family of secreted proteins and functions in head induction  
during *Xenopus* embryogenesis, acting as a potent inhibitor of Wnt signaling. (TREMBLNEW-  
ACC:AAF02678) (Table 13D).

**Table 13C.** BLASTN of FCTR13 with GenBank V07910

>patn:V07910 Human cysteine-rich secreted protein-like-N cDNA - Homo sapiens, 928 bp.  
Score = 1652 (247.9 bits), Expect = 1.6e-123, Sum P(2) = 1.6e-123  
Identities = 356/388 (91%), Positives = 356/388 (91%), Strand = Plus / Plus

```

Query:      1  ATCGGGCATCGCTGGTCTGCTGCTGCTCCTCTGCGCTGGTGACCTCCTTCACTGCA  60
             ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct:     105  AGCGCGCATCTGCTGGTCTGCTGCTGCTCCTCTCTACCTGGTGATCCCTCCGCTGCA  164

Query:      61  GCCTCTATCCACGATGCTCATGCCCAAGAGAGCTCCTTGGGTCTTACAGGCCTCAGAGC  120
             ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct:     165  GCTCCTATCCATGATGCTGACGCCCAAGAGAGCTCCTTGGGTCTTACAGGCCTCAGAGC  224

Query:     121  CTACTCCAAGGCTTCAGCGACTTTTCCTGAAAGATGACCTGCTTCGGGGCATAGACAGC  180
             ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct:     225  CTACTCCAAGGCTTCAGCGACTTTTCCTGAAAGGTAACCTGCTTCGGGGCATAGACAGC  284

Query:     181  TTCTTCTCTGCCCCCATGGACTTCCGGGGCTCCCTAGGAACCTACCAAGAGGAGAAC  240
             ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct:     285  TTATCTCTCCGCCCATGGACTTCCGGGGCTCCCTGGGAACCTACCAAGAGGAGAAC  344

Query:     241  GAGGAGCACCGAGCTGAGGAACAACCCCTCTCAGGCCACTCCATATTGACAAGGTGACC  300
             ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct:     345  CAGGAGCACCGAGCTGGGGAACAACCCCTCTCAGGCCACTCAGATCGACAAGATGACC  404

Query:     301  GACAAATAGACAGGAGAGTGCTGATCTCCGAGAAGTGTTGGCATCCATCCAGCCGGCG  360
             ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct:     405  GACAACAGACAGGAGAGTGCTGATCTCCGAGAATGTGGTCATCCATTCAACACGCG  464

Query:     361  GAGGGGAGCTTCGAGGGTAACGGAAAG  388
             ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct:     465  GAGGGGAGCTTCGAGGGTGATTGAAGG  492 (SEQ ID NO:64)

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Query:      384  GAAGGCGGCGCCCTGGTGTGCATTCGGAAGGCTATGGACAACCTTCCATGCAAGAGCTCCA 443
Sbjct:      506  GGAGAAAGAGGCGCTTGTTACCATCCAGAAGGCCACGACAGCTTCCACACAGAATCCA 565

Query:      444  TCCCGGGGTGGCCTTTTGATCATGAAGCTGCGCGGTGGAGGTCCACCAACAATGTCCT 503
Sbjct:      566  TCCCGGGGTGGCCTTCTGGATCATTAAGCTGCGCAGGCGGAGGTCCACCGAGGATGCGCT 625

Query:      504  GGAGGCGGCGCCGCTGGCTCAGTGAGAAGCGACACCGCCTGCAGGCCATCCAGGATGGGCT 563
Sbjct:      626  GGAGGCGGCGCACTGGCTCAGCGAGAAGCGACACCGCTGCAGGCCATCCGGGATGGACT 685

Query:      564  CCACGAGGGGACCGCGGAGGACGCTCTAAAGAGAGGGGACCCAGGCTCTCTCCCACTCGG 623
Sbjct:      686  CCACGAGGGGACCAACGACGAGGCTCTAGAGAGAGGGGACCGAGAGCTCTCTCCCACTCAG 745

Query:      624  GCTGTCTCTTC-GAAGAACCACTTACTGTACATCTTCAGGCTTTCCTGGCAGCTATAGGG 682
Sbjct:      746  GCTGTCCCCCGAAGAACCACTTACTGTATCTCTCAGGCGCTCTCGGCAGCTGTAGGG 805

Query:      683  GTTGGGACCGGGGAGCACTCTG 704
Sbjct:      806  GTGGGGACCGGGGAGCACTCTG 827 (SEQ ID NO:65)

```

ptntr:TREMBLNEW-ACC:AF02678 SOGGY-1 PROTEIN - Homo sapiens (Human), 242 aa. Dickkopf-1 (dkk-1) is member of a novel family of secreted proteins and functions in head induction during *Xenopus* embryogenesis, acting as a potent inhibitor of Wnt signaling. Score = 813 (286.2 bits), Expect = 6.3e-84, Sum P(2) = 6.3e-84 Identities = 166/218 (76%), Positives = 181/218 (83%), Frame = +1

```
Query:      4 RHPVLVLLLLSALVTSPTAASIHDAHAQESSLGLTGLQLQQFGRFLPKDLLRGIDSF 183
           |||||+|||||+|||||+|||||+|||||+|||||+|||||+|||||+
Sbjct:     12 RHLLVLLLLSTLVIPSAAPAIHDADAQESSLGLTGLQLQQFGRFLPKGKLRGIDSF 71

Query:     184 FSAPMDFRGLPRNYQEENEHQLRNNTSSHLHKVDNKTGEVLISEKVVASIQPAE 363
           |||||+|||||+|||||+|||||+|||||+|||||+|||||+|||||+
Sbjct:     72 FSAPMDFRGLPGNYHKEENQEQHQLGNNLTLSHLQIDKNMTKTGEVLISENVVASIQPAE 131

Query:     364 GSFEGNWKA-----ALVSIRKAMDNFHAELHPRVAFWIMKLPRWRSHHNVLGGRWLS 525
           |||||+|||||+|||||+|||||+|||||+|||||+|||||+|||||+
Sbjct:    132 GSFEGDLKVPMEEKALVP IQKATDS FHTBLHPRVAFWI I KLPRRRSHQDALEGGHWLS 191

Query:     526 EKRRLQAIQDGLHEGTREDVLKEGTQGSSSHGLSSER 639
           |||||+|||||+|||||+|||||+|||||+|||||+|||||+|||||+
Sbjct:    192 EKRRHLQAIRDGLRKGTGHKDVLEGTESSSHSRSLRP * 229 (SEO ID NO:66)
```

Score = 54 (19.0 bits), Expect = 6.3e-84, Sum P(2) = 6.3e-84  
Identities = 12/15 (80%), Positives = 12/15 (80%), Frame = +3

Query: 633 RKTHLLYIFRLSWQL 677  
 ||||| | |  
 Sbjct: 228 RKTHLLYILRPSROL 242 (SEQ ID NO:67)

37

(gi|10644567| gb|AAG21340.1| AF274312\_1 (AF274312) soggy precursor [Mus musculus]) (SEQ ID NO:69) on line 3, and mouse Soggy-1 protein (gi|10644569| gb|AAG21341.1| AF274313\_1 (AF274313) soggy precursor [Mus musculus]) (SEQ ID NO:70) on line 4. Table 13E depicts a ClustalW alignment of FCTR13 against proteins from a public database. Based on this alignment, black outlined amino acid residues indicate regions of conserved sequence (i.e., regions that may be required to preserve structural or functional properties); grayed amino acid residues can be mutated to a residue with comparable steric and/or chemical properties without altering protein structure or function (e.g. L to V, I, or M); non-highlighted amino acid residues can potentially be mutated to a much broader extent without altering structure or function.

**Table 13E.** ClustalW alignment of the FCTR13.

10	20	30	40	50	60	
FCTR13	.....	.....	.....	.....	.....	.....
Line 2	.....	.....	.....	.....	.....	.....
Line 3	.....	.....	.....	.....	.....	.....
Line 4	.....	.....	.....	.....	.....	.....
20	70	80	90	100	110	120
FCTR13	.....	.....	.....	.....	.....	.....
Line 2	.....	.....	.....	.....	.....	.....
Line 3	.....	.....	.....	.....	.....	.....
Line 4	.....	.....	.....	.....	.....	.....
25	130	140	150	160	170	180
FCTR13	.....	.....	.....	.....	.....	.....
Line 2	.....	.....	.....	.....	.....	.....
Line 3	.....	.....	.....	.....	.....	.....
Line 4	.....	.....	.....	.....	.....	.....
30	190	200	210	220	230	240
FCTR13	.....	.....	.....	.....	.....	.....
Line 2	.....	.....	.....	.....	.....	.....
Line 3	.....	.....	.....	.....	.....	.....
Line 4	.....	.....	.....	.....	.....	.....
40	250	260	270	280	290	300
FCTR13	.....	.....	.....	.....	.....	.....
Line 2	.....	.....	.....	.....	.....	.....
Line 3	.....	.....	.....	.....	.....	.....
Line 4	.....	.....	.....	.....	.....	.....
45	310	320				
FCTR13	.....	.....				
Line 2	.....	.....				
Line 3	.....	.....				
Line 4	.....	.....				

From these analyses, it is seen that the FCTR13 AL121723\_A A nucleic acid and protein have a strong similarity with human soggy-1 protein.

The nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

### FCTR14 (AL121756\_A)

The novel nucleic acid encoding a novel secreted protein is shown in Table 14A. This sequence contains an initiation codon at the 5' end, and a TGA stop codon was identified at the 3' end indicating that this sequence is a coding sequence. The start and stop codons are shown in bold type. This sequence originates in chromosome 20 clone RP4-726C3.

**Table 14A.** FCTR14 (AL121756\_A) nucleotide sequence (SEQ ID NO:27).

**atg**ctgcgggactcctgtgcttggcactctgcagcctgctgactggcagcgagctgaccctggggcactgc  
tgcgggttgggcatcctggacatcatgaaccctgaggtccagagccatggatgagagtcatacctggagaa  
gatggcagcgcgagggcaggcaagaacacagccagggatgaacacctatcaagggcatcaccaatttgaaggtg  
aaggtatgctccagctgcccgtcatcacactgaaccttctgacctggagtgggcatcttccaattgtgtgtcca  
caggcatgaccgtcactggcaagagcttcatgggagggaaacatggagatcatcgtggccctgaacatcac  
agccaccacacccgcttctcggggatgaggagacagggcctccccctgttccaagatgagggtctgtgaggtc  
atccctgltcaatgtgaagataacctgcttagcaacatgctccccaagatggtcaacaagttcctggaca  
gcaccctgcacaaagtctcctctgggctgatgtgtcccgccatcgatgcagtcctggtgtatgtgaacag  
gaagtggaccacacctcagtcagcccatgctgtgggagcagatgggacccgtcaaatatgttctgatgtcc  
gcaccagccaccacagccagctacatccaactggacttcagtcctgtggtgacgagcgaaggggcaaaa  
ccatcaagcttctgtgatgcggggaggccctcaggttccccctgaggggttatgcaaaagggctcgtcgcagct  
gctgctccagcaccctcctctctgcagagcttgcctctctgcagagcttcttcaatgtgaatatccag  
gatacaatgatgtgtgagctgccccacacaccccaagacccctggctcgttcttctcctgaagtgggtg  
tagcttatcccaagtcacaagcccttgacgaccagatcaagataaagaagccctccaaggtcactatgaa  
gacaggccaagagcctgctgcacctccacagaccctggagatgttcgcagctcggtgcggagcgaagct  
ccaatgtccctcttctcctagaagtgcacttcaatctgaaggtccagtcactcagtcgcatgagaaccagc  
tgagatggccacttcttggacagattactgagcttcttccggagagtcctcactcattgtggcaacttca  
tgagagggaattaaatggcttctcaccagctatctcgaagaagcctacatccagttgtcaatgatgtg  
cttcaagttggggtccactcccgacttcttgccatgaattacaacctggctgagctggacatagtag  
agcttgggggcatcatggaacctgcccacat**tga**

The encoded protein is presented using the one-letter code in Table 14B. The protein has a moderate probability of being sorted to the plasma membrane. A signal peptide most likely is cleaved between residues 18 and 19, *i.e.*, at the dash in the amino acid sequence TRA-DPG.

**Table 14B.** Encoded FCTR14 protein sequence (SEQ ID NO:28).

MLRILCLALCSLLTGTTRADPGALLRLGMDIMNREVQSAMDESHILEKMAAEAGKKQFGMKPIKIGITNLKV  
KDVLQPLVITLNFVPGVGIPQCSTGMTVTGKSFMGCGNMEIIVALNITATNRLRLDEETGLPVFKSEGCEV  
ILVNVKTNLPSNMLPKMNVLTSLHKVLPGLMCPAIDAVLVYVNRKVTNLSDDPMFVGQMGTVKTVLMS  
APATTASYIQLDPSVPUVQQKGKTKLADAGEALTFFPEGYAKGSSQLLLPATPLSAELALLQKSFHVNIQ  
DTMIGGLPPQTTKTLARFIPEVAVAYPKSKPLTTQIKIKKPKVTMTKGSLLLHLSTLEMFARWRKA  
PMSLFLLEVHFNLVQVYSVHENQLQMATSLDRLLSLSRKSSSIGNFNRELGTGITSYLEEAYIPVNVNDV  
LQVGLPLDPLAMNLYNAELDIVELGGMPEADI

A BLASTN search of sequence databases for the FCTR14 nucleic acid sequence identified significant similarities to the human genomic clone HSDJ726C3, isolated from human DNA sequence from clone RP4-726C3 on chromosome 20. In a BLASTX comparison, it was

found that the full FCTR14 amino acid sequence has 130 of 391 residues (33%), are identical to, and 229 of 391 residues (58%) positive with, rat potential ligand-binding protein RY2G5 having a total of 409 amino acid residues (SPTREMBL-ACC:Q05704) (SEQ ID NO:71). The BLASTX alignment is shown in Table 14C.

**Table 14C.** BLASTX alignment of FCTR14

```
>ptnr:SPTREMBL-ACC:Q05704 POTENTIAL LIGAND-BINDING PROTEIN RY2G5 - RATTUS NORVEGICUS
(RAT), 470 aa (fragment).
CC -!- TISSUE SPECIFICITY: SUBREGIONS OF THE OLFACTORY MUCOSA.

Score = 579 (203.8 bits), Expect = 2.0e-55, P = 2.0e-55
Identities = 130/391 (33%), Positives = 229/391 (58%), Frame = +1

Query: 175 MKPIKGIITNLKVKVDQLPVITLNFVPGVGIFQCVSTGMTVTGKFSMGNGMEIIVALNITA 354
      + +++++|++ ++| +++++ +|+|++ +| + +| +| +| +| +| +| +|
Sbjct: 73 LSTVQIGTGLRIVELTLPVSVRLPLPGVYLSLYTRVAINGKSLIGF-LDIAVEVNITA 131

Query: 355 TNRLRLDEETGLPVFKSEGCEVILNVNKTNLPSNMLPKMKNFLODSTLHKVLPLGMLCPAI 534
      ||| | | | | | + +| +| +| +| +| +| +| +| +| +| +| +| +|
Sbjct: 132 KVRLLTMDR-TGYPRLVIERCDTLGGIKVKLLRGLLNLVDNLVNRVLANVLPDLLCPIV 190

Query: 535 DAVLVYVNRKWTNLSDPMPVPGMGTVKYVLSMAPATTASYIQLDFSPVQQQKGKTIKLA 714
      ||| | | | + + + +| +| +| +| +| +| +| +| +| +| +| +| +|
Sbjct: 191 DVVLGLVNDQLGLVDSLVLGILGSVQYTFSSLPVLTGTFLELNLNTLVGEAGGLIDYP 250

Query: 715 DAGEALT---PPEGYAKG---SSQLLPATFLSAELALLQK--SFHVNIQDITMIGELPP 867
      | + | | | | | | | +| +| +| +| +| +| +| +| +| +| +| +|
Sbjct: 251 LGRPAMLRPPQMPPLPMGDNTNSQLAISANFLSSSVLTMLQKQALDIDITDGMFEDLPP 310

Query: 868 QTTKTLARFIEPVAVYPKSKPLTTQIKIKKPKVTKMTKGLSLHLHSTLEMFARWRSK 1047
      ||| | | | | | +| +| +| +| +| +| +| +| +| +| +| +| +|
Sbjct: 311 LTTSTLGALIPKVFQQYPESRPLTIRIQVNPPTVTLQKDKALVKVFATSEVVVSQ-PND 369

Query: 1048 APMSLELLEVHFNLKVQYPSVHENQOMATSLDRLLSLSRKSSSIGNFNERELTGPITSYL 1227
      ++ |+++| +| +| +| +| +| +| +| +| +| +| +| +| +| +| +|
Sbjct: 370 VETTICLIDVDTDLLASFVSGDKLMDAKLDKT-SLNLRTSNVGNFVDFILEMLVEKIF 428

Query: 1228 EEAYIPVVDVNLQVGLPLPDLFLAMNYNLAELDIVE 1332
      + |++| +| +| +| +| +| +| +| +| +| +| +| +| +| +| +|
Sbjct: 429 DLAFMPAMNAILGSGVPLPKILNIDFSNADIDVLE 463
```

A multiple sequence alignment for FCTR14 AL121756\_A is given in Table 14D, with the protein of the invention being shown on line 3, in a ClustalW analysis comparing the protein of the invention with related protein sequences. Table 14D depicts a ClustalW alignment of FCTR13 with proteins from the public database. The alignment is presented against Q05704 - POTENTIAL LIGAND-BINDING PROTEIN RY2G5 (FRAGMENT) (SEQ ID NO:71) and Q05701 - POTENTIAL LIGAND-BINDING PROTEIN RYA3 (SEQ ID NO:72). Based on this alignment, black outlined amino acid residues indicate regions of conserved sequence (i.e., regions that may be required to preserve structural or functional properties); grayed amino acid residues can be mutated to a residue with comparable steric and/or chemical properties without altering protein structure or function (e.g. L to V, I, or M); non-highlighted amino acid residues can potentially be mutated to a much broader extent without altering structure or function.



**TABLE 14D** ClustalW alignment of FCTR14 (AL121756\_A) protein

5	Q05704_RY2G5_RAT	RLHRRRPRPGEIPASVATGALGPGGGLGTGS--ILANSGILAGCGGLGCG-----
	Q05701_RYA3_RAT	--MPPQVYALLLWGLATPCLGLETVTSTLARDKDKDEGKASQNSLVGGPILQNVLTGTVT
	AL121756_A	--LRRLCLACCSLTGTTRADPGALLRLAMD--MNRREVQSAMDESHLEK-----
10	Q05704_RY2G5_RAT	---GGLGCGGGLGGGVLGMDGEGGGLSVVQGITGLFVELTLPFVSVELLPVGVMVLS
	Q05701_RYA3_RAT	SVNQSLGAGGLGGG---GLSYGGLFSVVELSLKIGELTLPFVSVELLPVGVMVLS
	AL121756_A	---MAREK--KKQFG-----MK-PKSGTNLKVQVGLFVITLINEVGVGVLFGCC
15	Q05704_RY2G5_RAT	LYRFAVAINKGS--LTFGLTIAVEVNITAKVRLTNR--TGYRVEVERODITLGGHVRKLR
	Q05701_RYA3_RAT	---HPRVBERGSGGPIGSLTQTAKEVNVSSKVALQNSP--RQDPLILTRCNILGCG--HISIT
	AL121756_A	YSYQMTWTGKSPMGQNMETVALNITATNRLLRDETCGLVFKSGECVVLVNVKTNIPS
20	Q05704_RY2G5_RAT	GLLENLVNENLVNVRVIANVLPDLCPVIVVGLVAVNDOLGLVDSVPLGILGSSVQPTSSSL
	Q05701_RYA3_RAT	SLLPETPFVGLVQVOTITKVLPGLLCFVVSLSVSNVNEELGATISLVPLGELSGVETFLAG
	AL121756_A	NMLRKKLNKFLDSTPLHKVLFGLYCFATPAVIVVNVNRKWNISDPMVWGQMTVRVIVMSA
25	Q05704_RY2G5_RAT	PLVTSSEFELDENLTVEAGGDEITVPLGRPAMLRPQMPPEIPMGNGNNSQTAASANTF
	Q05701_RYA3_RAT	PLHSNQVIELDNNPDKSISAGDMIDPKPR---LR-----MKMPKKECHT--SQVTVGLYLF
	AL121756_A	PATTSYVITLDFSPWQQQCKTKRLADAG-----EALTFPEGYAGSSQILLPATTF
30	Q05704_RY2G5_RAT	SSVITNLCQKGLDHDITGPFELPLPLTSTTSLALIPKVPQCVPSREPLTKRQVNPFP
	Q05701_RYA3_RAT	NVVEGLCTNGALDHDITPEVMPRNIPPLTIDLAALFESALGKIFPGQHLLSERVMKSE
	AL121756_A	SABLALQSG--FHWNTQTMHGEPLPCHRTLRFPIEVAVAVTPKSEPLTCTCKIKRPF
35	Q05704_RY2G5_RAT	TVTLCKDRALVKVFAISEVWVSQF--MDVETTCITLIDTDTLBSFSVEGDKLMDAMLDK
	Q05701_RYA3_RAT	MITLCKNRKVTSTPVTTHLSSVF--QGTVALEQMGNVMTNHLVLPSTTKLSTLSLSEK
	AL121756_A	KVIMKTGSLHLHSTIDEMFAARWRSKAPMSLELLEHFNLRKVQVSVHENQMATSLDR
40	Q05704_RY2G5_RAT	-TSINRQSNVNEFDPFLEMLVKEKTFDLAMPAMATIGSGVPLPKILNDRSNAGIEV
	Q05701_RYA3_RAT	-LTVGLASSFSQPFDAASRBEWLSDWVRAAYCKKNEHELVGTHPLPKILNDRSNAGIEV
	AL121756_A	LLSISKSSSSNENEREITGFTSYLSEAYEIVVNDVLCVGEPLDEDLAMPAMATIGH
45	Q05704_RY2G5_RAT	I--DILL--ST--- (SEQ ID NO: 71)
	Q05701_RYA3_RAT	I--NAV--TV--P- (SEQ ID NO: 72)
	AL121756_A	V--GG--MEP--DI (SEQ ID NO: 28)

Finally, FCTR14 was found to have high homology to the domains shown in Table 14D.

**Table 14D:** CD domain analysis of FCTR14

Sequences producing significant alignments:	Score (bits)	E value
BPI/LBP/CETP C-terminal domain; Bactericidal permeability-incr...	72.8	4e-14
BPI/LBP/CETP N-terminal domain; Bactericidal permeability-incr...	60.8	1e-10
LBP_BPI_CETP, LBP / BPI / CETP family	45.8	5e-06

The FCTR14 nucleic acids and proteins of the invention are potentially useful in the treatment of cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders.

### FCTR1X Nucleic Acids and Polypeptides

One aspect of the invention pertains to isolated nucleic acid molecules that encode FCTR1X polypeptides or biologically-active portions thereof. Also included in the invention are nucleic acid fragments sufficient for use as hybridization probes to identify FCTR1X-encoding nucleic acids (e.g., FCTR1X mRNAs) and fragments for use as PCR primers for the amplification

and/or mutation of FCTRX nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule may be single-stranded or double-stranded, but preferably is comprised double-stranded DNA.

An FCTRX nucleic acid can encode a mature FCTRX polypeptide. As used herein, a "mature" form of a polypeptide or protein disclosed in the present invention is the product of a naturally occurring polypeptide or precursor form or proprotein. The naturally occurring polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an open reading frame described herein. The product "mature" form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps as they may take place within the cell, or host cell, in which the gene product arises. Examples of such processing steps leading to a "mature" form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an open reading frame, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine, would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a "mature" form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or a combination of any of them.

The term "probes", as utilized herein, refers to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as approximately, e.g., 6,000 nt, depending upon the specific use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are generally obtained from a natural or recombinant source, are highly specific, and much slower to hybridize than shorter-length oligomer probes. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

The term "isolated" nucleic acid molecule, as utilized herein, is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid.

Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5'- and 3'-termini of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated FCTR<sub>X</sub> nucleic acid molecules can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell/tissue from which the nucleic acid is derived (*e.g.*, brain, heart, liver, spleen, etc.). Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or a complement of this aforementioned nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29 as a hybridization probe, FCTR<sub>X</sub> molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL 2<sup>nd</sup> Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to FCTR<sub>X</sub> nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment of the invention, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or

a complement thereof. Oligonucleotides may be chemically synthesized and may also be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or a portion of this nucleotide sequence (e.g., a fragment that can be used as a probe or primer or a fragment encoding a biologically-active portion of an FCTR polypeptide). A nucleic acid molecule that is complementary to the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, is one that is sufficiently complementary to the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, thereby forming a stable duplex.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, van der Waals, hydrophobic interactions, and the like. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type. Homologs are nucleic acid sequences or amino acid sequences of a particular gene that are derived from different species.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or

analogues of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 70%, 80%, or 95% identity (with a preferred identity of 80-95%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of FCTR<sub>X</sub> polypeptides. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the invention, homologous nucleotide sequences include nucleotide sequences encoding for an FCTR<sub>X</sub> polypeptide of species other than humans, including, but not limited to: vertebrates, and thus can include, *e.g.*, frog, mouse, rat, rabbit, dog, cat, cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the exact nucleotide sequence encoding human FCTR<sub>X</sub> protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, as well as a polypeptide possessing FCTR<sub>X</sub> biological activity. Various biological activities of the FCTR<sub>X</sub> proteins are described below.

An FCTR<sub>X</sub> polypeptide is encoded by the open reading frame ("ORF") of an FCTR<sub>X</sub> nucleic acid. An ORF corresponds to a nucleotide sequence that could potentially be translated into a polypeptide. A stretch of nucleic acids comprising an ORF is uninterrupted by a stop codon. An ORF that represents the coding sequence for a full protein begins with an ATG "start" codon and terminates with one of the three "stop" codons, namely, TAA, TAG, or TGA. For the purposes of this invention, an ORF may be any part of a coding sequence, with or without a start codon, a stop codon, or both. For an ORF to be considered as a good candidate for coding for a *bona fide* cellular protein, a minimum size requirement is often set, *e.g.*, a stretch of DNA that would encode a protein of 50 amino acids or more.

The nucleotide sequences determined from the cloning of the human FCTR<sub>X</sub> genes allows for the generation of probes and primers designed for use in identifying and/or cloning FCTR<sub>X</sub> homologues in other cell types, *e.g.* from other tissues, as well as FCTR<sub>X</sub> homologues from other vertebrates. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 consecutive sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29; or an anti-sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29; or of a naturally occurring mutant of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29.

Probes based on the human FCTR<sub>X</sub> nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.* the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissues which mis-express an FCTR<sub>X</sub> protein, such as by measuring a level of an FCTR<sub>X</sub>-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting FCTR<sub>X</sub> mRNA levels or determining whether a genomic FCTR<sub>X</sub> gene has been mutated or deleted.

"A polypeptide having a biologically-active portion of an FCTR<sub>X</sub> polypeptide" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically-active portion of FCTR<sub>X</sub>" can be prepared by isolating a portion of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, that encodes a polypeptide having an FCTR<sub>X</sub> biological activity (the biological activities of the FCTR<sub>X</sub> proteins are described below), expressing the encoded portion of FCTR<sub>X</sub> protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of FCTR<sub>X</sub>.

### FCTR<sub>X</sub> Nucleic Acid and Polypeptide Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, due to degeneracy of the genetic code and thus encode the same FCTR<sub>X</sub> proteins as that encoded by the nucleotide sequences shown in SEQ ID NO NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29. In another embodiment, an isolated nucleic acid molecule of the invention has a

nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30.

In addition to the human FCTR<sub>X</sub> nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the FCTR<sub>X</sub> polypeptides may exist within a population (e.g., the human population). Such genetic polymorphism in the FCTR<sub>X</sub> genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame (ORF) encoding an FCTR<sub>X</sub> protein, preferably a vertebrate FCTR<sub>X</sub> protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the FCTR<sub>X</sub> genes. Any and all such nucleotide variations and resulting amino acid polymorphisms in the FCTR<sub>X</sub> polypeptides, which are the result of natural allelic variation and that do not alter the functional activity of the FCTR<sub>X</sub> polypeptides, are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding FCTR<sub>X</sub> proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the FCTR<sub>X</sub> cDNAs of the invention can be isolated based on their homology to the human FCTR<sub>X</sub> nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500, 750, 1000, 1500, or 2000 or more nucleotides in length. In yet another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding FCTR<sub>X</sub> proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T<sub>m</sub>) for the specific sequence at a defined ionic strength and pH. The T<sub>m</sub> is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at T<sub>m</sub>, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at

pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (e.g., 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions are hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C, followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS



at 37°C. Other conditions of moderate stringency that may be used are well-known within the art. See, *e.g.*, Ausubel, *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990; GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel, *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981. *Proc Natl Acad Sci USA* 78: 6789-6792.

#### ***Conservative Mutations***

In addition to naturally-occurring allelic variants of FCTR X sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, thereby leading to changes in the amino acid sequences of the encoded FCTR X proteins, without altering the functional ability of said FCTR X proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequences of the FCTR X proteins without altering their biological activity, whereas an "essential" amino acid residue is required for such biological activity. For example, amino acid residues that are conserved among the FCTR X proteins of the invention are predicted to be particularly non-amenable to alteration. Amino acids for which conservative substitutions can be made are well-known within the art.

Another aspect of the invention pertains to nucleic acid molecules encoding FCTR X proteins that contain changes in amino acid residues that are not essential for activity. Such FCTR X proteins differ in amino acid sequence from SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein

comprises an amino acid sequence at least about 45% homologous to the amino acid sequences of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30. Preferably, the protein encoded by the nucleic acid molecule is at least about 60% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30; more preferably at least about 70% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30; still more preferably at least about 80% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30; even more preferably at least about 90% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30; and most preferably at least about 95% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30.

An isolated nucleic acid molecule encoding an FCTR<sub>X</sub> protein homologous to the protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted, non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain.

Families of amino acid residues having similar side chains have been defined within the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted non-essential amino acid residue in the FCTR<sub>X</sub> protein is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an FCTR<sub>X</sub> coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for FCTR<sub>X</sub> biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

The relatedness of amino acid families may also be determined based on side chain interactions. Substituted amino acids may be fully conserved "strong" residues or fully

conserved "weak" residues. The "strong" group of conserved amino acid residues may be any one of the following groups: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW, wherein the single letter amino acid codes are grouped by those amino acids that may be substituted for each other. Likewise, the "weak" group of conserved residues may be any one of the following: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY, wherein the letters within each group represent the single letter amino acid code.

In one embodiment, a mutant FCTR protein can be assayed for (i) the ability to form protein:protein interactions with other FCTR proteins, other cell-surface proteins, or biologically-active portions thereof, (ii) complex formation between a mutant FCTR protein and an FCTR ligand; or (iii) the ability of a mutant FCTR protein to bind to an intracellular target protein or biologically-active portion thereof; (e.g. avidin proteins).

In yet another embodiment, a mutant FCTR protein can be assayed for the ability to regulate a specific biological function (e.g., regulation of insulin release).

### Antisense Nucleic Acids

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein (e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence). In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire FCTR coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of an FCTR protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30; or antisense nucleic acids complementary to an FCTR nucleic acid sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an FCTR protein. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding the FCTR protein. The term "noncoding region" refers to 5' and 3' sequences which flank the

coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding the FCTR<sub>X</sub> protein disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of FCTR<sub>X</sub> mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of FCTR<sub>X</sub> mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of FCTR<sub>X</sub> mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally-occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids (*e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used).

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an FCTR<sub>X</sub> protein to thereby inhibit expression of the protein (*e.g.*, by

inhibiting transcription and/or translation). The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface (e.g., by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens). The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient nucleic acid molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other. See, e.g., Gaultier, *et al.*, 1987. *Nucl. Acids Res.* **15**: 6625-6641. The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (see, e.g., Inoue, *et al.* 1987. *Nucl. Acids Res.* **15**: 6131-6148) or a chimeric RNA-DNA analogue (see, e.g., Inoue, *et al.*, 1987. *FEBS Lett.* **215**: 327-330).

### **Ribozymes and PNA Moieties**

Nucleic acid modifications include, by way of non-limiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In one embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes as described in Haselhoff and Gerlach 1988. *Nature* 334: 585-591) can be used to catalytically cleave FCTR mRNA transcripts to thereby inhibit translation of FCTR mRNA. A ribozyme having specificity for an FCTR-encoding nucleic acid can be designed based upon the nucleotide sequence of an FCTR cDNA disclosed herein (i.e., SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide

sequence of the active site is complementary to the nucleotide sequence to be cleaved in an FCTR<sub>X</sub>-encoding mRNA. See, e.g., U.S. Patent 4,987,071 to Cech, *et al.* and U.S. Patent 5,116,742 to Cech, *et al.* FCTR<sub>X</sub> mRNA can also be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, FCTR<sub>X</sub> gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the FCTR<sub>X</sub> nucleic acid (e.g., the FCTR<sub>X</sub> promoter and/or enhancers) to form triple helical structures that prevent transcription of the FCTR<sub>X</sub> gene in target cells. See, e.g., Helene, 1991. *Anticancer Drug Des.* 6: 569-84; Helene, *et al.* 1992. *Ann. N.Y. Acad. Sci.* 660: 27-36; Maher, 1992. *Bioassays* 14: 807-15.

In various embodiments, the FCTR<sub>X</sub> nucleic acids can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids. See, e.g., Hyrup, *et al.*, 1996. *Bioorg Med Chem* 4: 5-23. As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics (e.g., DNA mimics) in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup, *et al.*, 1996. *supra*; Perry-O'Keefe, *et al.*, 1996. *Proc. Natl. Acad. Sci. USA* 93: 14670-14675.

PNAs of FCTR<sub>X</sub> can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, e.g., inducing transcription or translation arrest or inhibiting replication. PNAs of FCTR<sub>X</sub> can also be used, for example, in the analysis of single base pair mutations in a gene (e.g., PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, e.g., S<sub>1</sub> nucleases (see, Hyrup, *et al.*, 1996. *supra*); or as probes or primers for DNA sequence and hybridization (see, Hyrup, *et al.*, 1996, *supra*; Perry-O'Keefe, *et al.*, 1996. *supra*).

In another embodiment, PNAs of FCTR<sub>X</sub> can be modified, e.g., to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of FCTR<sub>X</sub> can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes (e.g., RNase H and DNA polymerases) to interact with the DNA portion while the PNA portion

would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (*see*, Hyrup, *et al.*, 1996. *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup, *et al.*, 1996. *supra* and Finn, *et al.*, 1996. *Nucl Acids Res* 24: 3357-3363. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA. *See, e.g.*, Mag, *et al.*, 1989. *Nucl Acid Res* 17: 5973-5988. PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment. *See, e.g.*, Finn, *et al.*, 1996. *supra*. Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. *See, e.g.*, Petersen, *et al.*, 1975. *Bioorg. Med. Chem. Lett.* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (*see, e.g.*, Letsinger, *et al.*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86: 6553-6556; Lemaitre, *et al.*, 1987. *Proc. Natl. Acad. Sci.* 84: 648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (*see, e.g.*, PCT Publication No. WO 89/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (*see, e.g.*, Krol, *et al.*, 1988. *BioTechniques* 6:958-976) or intercalating agents (*see, e.g.*, Zon, 1988. *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, and the like.

### **FCTRX Polypeptides**

A polypeptide according to the invention includes a polypeptide including the amino acid sequence of FCTRX polypeptides whose sequences are provided in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residues shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, while still encoding a protein that maintains its FCTRX activities and physiological functions, or a functional fragment thereof.

In general, an FCTRX variant that preserves FCTRX-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from

the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

One aspect of the invention pertains to isolated FCTR<sub>X</sub> proteins, and biologically-active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-FCTR<sub>X</sub> antibodies. In one embodiment, native FCTR<sub>X</sub> proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, FCTR<sub>X</sub> proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, an FCTR<sub>X</sub> protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the FCTR<sub>X</sub> protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of FCTR<sub>X</sub> proteins in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly-produced. In one embodiment, the language "substantially free of cellular material" includes preparations of FCTR<sub>X</sub> proteins having less than about 30% (by dry weight) of non-FCTR<sub>X</sub> proteins (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-FCTR<sub>X</sub> proteins, still more preferably less than about 10% of non-FCTR<sub>X</sub> proteins, and most preferably less than about 5% of non-FCTR<sub>X</sub> proteins. When the FCTR<sub>X</sub> protein or biologically-active portion thereof is recombinantly-produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the FCTR<sub>X</sub> protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of FCTR<sub>X</sub> proteins in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of FCTR<sub>X</sub> proteins having less than about 30% (by dry weight) of chemical precursors or non-FCTR<sub>X</sub> chemicals, more preferably less than about 20% chemical precursors or non-FCTR<sub>X</sub> chemicals, still more preferably less than about 10% chemical precursors or non-FCTR<sub>X</sub> chemicals, and most preferably less than about 5% chemical precursors or non-FCTR<sub>X</sub> chemicals.



Biologically-active portions of FCTR<sub>X</sub> proteins include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequences of the FCTR<sub>X</sub> proteins (e.g., the amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30) that include fewer amino acids than the full-length FCTR<sub>X</sub> proteins, and exhibit at least one activity of an FCTR<sub>X</sub> protein. Typically, biologically-active portions comprise a domain or motif with at least one activity of the FCTR<sub>X</sub> protein. A biologically-active portion of an FCTR<sub>X</sub> protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acid residues in length.

Moreover, other biologically-active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native FCTR<sub>X</sub> protein.

In an embodiment, the FCTR<sub>X</sub> protein has an amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30. In other embodiments, the FCTR<sub>X</sub> protein is substantially homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, and retains the functional activity of the protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail, below. Accordingly, in another embodiment, the FCTR<sub>X</sub> protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, and retains the functional activity of the FCTR<sub>X</sub> proteins of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30.

#### *Determining Homology Between Two or More Sequences*

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (i.e., as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. See, Needleman and Wunsch, 1970. *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty

of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region.

#### *Chimeric and Fusion Proteins*

The invention also provides FCTRX chimeric or fusion proteins. As used herein, an FCTRX "chimeric protein" or "fusion protein" comprises an FCTRX polypeptide operatively-linked to a non-FCTRX polypeptide. An "FCTRX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an FCTRX protein (SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30), whereas a "non-FCTRX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the FCTRX protein, *e.g.*, a protein that is different from the FCTRX protein and that is derived from the same or a different organism. Within an FCTRX fusion protein the FCTRX polypeptide can correspond to all or a portion of an FCTRX protein. In one embodiment, an FCTRX fusion protein comprises at least one biologically-active portion of an FCTRX protein. In another embodiment, an FCTRX fusion protein comprises at least two biologically-active portions of an FCTRX protein. In yet another embodiment, an FCTRX fusion protein comprises at least three biologically-active portions of an FCTRX protein. Within the fusion protein, the term "operatively-linked" is intended to indicate that the FCTRX polypeptide and the non-FCTRX polypeptide are fused in-frame with one another. The non-FCTRX polypeptide can be fused to the N-terminus or C-terminus of the FCTRX polypeptide.

In one embodiment, the fusion protein is a GST-FCTR<sub>X</sub> fusion protein in which the FCTR<sub>X</sub> sequences are fused to the C-terminus of the GST (glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant FCTR<sub>X</sub> polypeptides.

In another embodiment, the fusion protein is an FCTR<sub>X</sub> protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of FCTR<sub>X</sub> can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is an FCTR<sub>X</sub>-immunoglobulin fusion protein in which the FCTR<sub>X</sub> sequences are fused to sequences derived from a member of the immunoglobulin protein family. The FCTR<sub>X</sub>-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between an FCTR<sub>X</sub> ligand and an FCTR<sub>X</sub> protein on the surface of a cell, to thereby suppress FCTR<sub>X</sub>-mediated signal transduction *in vivo*. The FCTR<sub>X</sub>-immunoglobulin fusion proteins can be used to affect the bioavailability of an FCTR<sub>X</sub> cognate ligand. Inhibition of the FCTR<sub>X</sub> ligand/FCTR<sub>X</sub> interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.* promoting or inhibiting) cell survival. Moreover, the FCTR<sub>X</sub>-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-FCTR<sub>X</sub> antibodies in a subject, to purify FCTR<sub>X</sub> ligands, and in screening assays to identify molecules that inhibit the interaction of FCTR<sub>X</sub> with an FCTR<sub>X</sub> ligand.

An FCTR<sub>X</sub> chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g.*, Ausubel, *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). An FCTR<sub>X</sub>-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the FCTR<sub>X</sub> protein.

### *FCTR X Agonists and Antagonists*

The invention also pertains to variants of the FCTR X proteins that function as either FCTR X agonists (*i.e.*, mimetics) or as FCTR X antagonists. Variants of the FCTR X protein can be generated by mutagenesis (*e.g.*, discrete point mutation or truncation of the FCTR X protein).

An agonist of the FCTR X protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the FCTR X protein. An antagonist of the FCTR X protein can inhibit one or more of the activities of the naturally occurring form of the FCTR X protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the FCTR X protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the FCTR X proteins.

Variants of the FCTR X proteins that function as either FCTR X agonists (*i.e.*, mimetics) or as FCTR X antagonists can be identified by screening combinatorial libraries of mutants (*e.g.*, truncation mutants) of the FCTR X proteins for FCTR X protein agonist or antagonist activity. In one embodiment, a variegated library of FCTR X variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of FCTR X variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential FCTR X sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of FCTR X sequences therein. There are a variety of methods which can be used to produce libraries of potential FCTR X variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential FCTR X sequences. Methods for synthesizing degenerate oligonucleotides are well-known within the art. *See, e.g.*, Narang, 1983. *Tetrahedron* 39: 3; Itakura, *et al.*, 1984. *Annu. Rev. Biochem.* 53: 323; Itakura, *et al.*, 1984. *Science* 198: 1056; Ike, *et al.*, 1983. *Nucl. Acids Res.* 11: 477.

### *Polypeptide Libraries*

In addition, libraries of fragments of the FCTR X protein coding sequences can be used to generate a variegated population of FCTR X fragments for screening and subsequent selection of variants of an FCTR X protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an FCTR X coding sequence with a

nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double-stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with  $S_1$  nuclease, and ligating the resulting fragment library into an expression vector. By this method, expression libraries can be derived which encodes N-terminal and internal fragments of various sizes of the FCTRX proteins.

Various techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of FCTRX proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify FCTRX variants. *See, e.g.,* Arkin and Yourvan, 1992. *Proc. Natl. Acad. Sci. USA* 89: 7811-7815; Delgrave, *et al.*, 1993. *Protein Engineering* 6:327-331.

## **Anti-FCTRX Antibodies**

The invention encompasses antibodies and antibody fragments, such as  $F_{ab}$  or  $(F_{ab})_2$ , that bind immunospecifically to any of the FCTRX polypeptides of said invention.

An isolated FCTRX protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind to FCTRX polypeptides using standard techniques for polyclonal and monoclonal antibody preparation. The full-length FCTRX proteins can be used or, alternatively, the invention provides antigenic peptide fragments of FCTRX proteins for use as immunogens. The antigenic FCTRX peptides comprises at least 4 amino acid residues of the amino acid sequence shown in SEQ ID NO NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, and encompasses an epitope of FCTRX such that an antibody raised against the peptide forms a specific immune complex with FCTRX. Preferably, the antigenic peptide comprises at least 6, 8, 10, 15, 20, or 30 amino acid residues. Longer antigenic peptides are sometimes preferable over shorter antigenic peptides, depending on use and according to methods well known to someone skilled in the art.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of FCTR<sub>X</sub> that is located on the surface of the protein (*e.g.*, a hydrophilic region). As a means for targeting antibody production, hydrophathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation (*see, e.g.*, Hopp and Woods, 1981. *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle, 1982. *J. Mol. Biol.* 157: 105-142, each incorporated herein by reference in their entirety).

As disclosed herein, FCTR<sub>X</sub> protein sequences of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically-active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically-binds (immunoreacts with) an antigen, such as FCTR<sub>X</sub>. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F<sub>ab</sub> and F<sub>(ab)<sub>2</sub></sub> fragments, and an F<sub>ab</sub> expression library. In a specific embodiment, antibodies to human FCTR<sub>X</sub> proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to an FCTR<sub>X</sub> protein sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30, or a derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, recombinantly-expressed FCTR<sub>X</sub> protein or a chemically-synthesized FCTR<sub>X</sub> polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against FCTR<sub>X</sub> can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of FCTR<sub>X</sub>. A monoclonal antibody

composition thus typically displays a single binding affinity for a particular FCTR<sub>X</sub> protein with which it immunoreacts. For preparation of monoclonal antibodies directed towards a particular FCTR<sub>X</sub> protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be

utilized. Such techniques include, but are not limited to, the hybridoma technique (*see, e.g., Kohler & Milstein, 1975. Nature 256: 495-497*); the trioma technique; the human B-cell hybridoma technique (*see, e.g., Kozbor, et al., 1983. Immunol. Today 4: 72*) and the EBV hybridoma technique to produce human monoclonal antibodies (*see, e.g., Cole, et al., 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96*). Human monoclonal antibodies may be utilized in the practice of the invention and may be produced by using human hybridomas (*see, e.g., Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030*) or by transforming human B-cells with Epstein Barr Virus *in vitro* (*see, e.g., Cole, et al., 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96*). Each of the above citations is incorporated herein by reference in their entirety.

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an FCTR<sub>X</sub> protein (*see, e.g., U.S. Patent No. 4,946,778*). In addition, methods can be adapted for the construction of F<sub>ab</sub> expression libraries (*see, e.g., Huse, et al., 1989. Science 246: 1275-1281*) to allow rapid and effective identification of monoclonal F<sub>ab</sub> fragments with the desired specificity for an FCTR<sub>X</sub> protein or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. *See, e.g., U.S. Patent No. 5,225,539*. Antibody fragments that contain the idiotypes to an FCTR<sub>X</sub> protein may be produced by techniques known in the art including, but not limited to: (i) an F<sub>(ab')<sub>2</sub></sub> fragment produced by pepsin digestion of an antibody molecule; (ii) an F<sub>ab</sub> fragment generated by reducing the disulfide bridges of an F<sub>(ab')<sub>2</sub></sub> fragment; (iii) an F<sub>ab</sub> fragment generated by the treatment of the antibody molecule with papain and a reducing agent; and (iv) F<sub>v</sub> fragments.

Additionally, recombinant anti-FCTR<sub>X</sub> antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Patent No. 4,816,567; U.S. Pat. No. 5,225,539; European Patent Application No. 125,023; Better, *et al., 1988. Science 240: 1041-1043*; Liu, *et al., 1987. Proc. Natl. Acad. Sci.*

USA 84: 3439-3443; Liu, *et al.*, 1987. *J. Immunol.* 139: 3521-3526; Sun, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura, *et al.*, 1987. *Cancer Res.* 47: 999-1005; Wood, *et al.*, 1985. *Nature* 314 :446-449; Shaw, *et al.*, 1988. *J. Natl. Cancer Inst.* 80: 1553-1559; Morrison(1985) *Science* 229:1202-1207; Oi, *et al.* (1986) *BioTechniques* 4:214; Jones, *et al.*, 1986. *Nature* 321: 552-525; Verhoeyan, *et al.*, 1988. *Science* 239: 1534; and Beidler, *et al.*, 1988. *J. Immunol.* 141: 4053-4060. Each of the above citations are incorporated herein by reference in their entirety.

In one embodiment, methods for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of an FCTRX protein is facilitated by generation of hybridomas that bind to the fragment of an FCTRX protein possessing such a domain. Thus, antibodies that are specific for a desired domain within an FCTRX protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Anti-FCTRX antibodies may be used in methods known within the art relating to the localization and/or quantitation of an FCTRX protein (e.g., for use in measuring levels of the FCTRX protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies for FCTRX proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds (hereinafter "Therapeutics").

An anti-FCTRX antibody (e.g., monoclonal antibody) can be used to isolate an FCTRX polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-FCTRX antibody can facilitate the purification of natural FCTRX polypeptide from cells and of recombinantly-produced FCTRX polypeptide expressed in host cells. Moreover, an anti-FCTRX antibody can be used to detect FCTRX protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the FCTRX protein. Anti-FCTRX antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine



fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

### **FCTR Recombinant Expression Vectors and Host Cells**

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an FCTR protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, *GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY* 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include

those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., FCTR proteins, mutant forms of FCTR proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of FCTR proteins in prokaryotic or eukaryotic cells. For example, FCTR proteins can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (i) to increase expression of recombinant protein; (ii) to increase the solubility of the recombinant protein; and (iii) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See, e.g., Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (see, e.g., Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the FCTR expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cervisiae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.).

Alternatively, FCTR can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, e.g., Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, 1987. *Genes Dev.* 1: 268-277), lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. Immunol.* 43: 235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.* 8: 729-733) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, 1983. *Cell* 33: 741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle,

1989. *Proc. Natl. Acad. Sci. USA* 86: 5473-5477), pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, 1990. *Science* 249: 374-379) and the  $\alpha$ -fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3: 537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively-linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to FCTRX mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes *see, e.g.*, Weintraub, *et al.*, "Antisense RNA as a molecular tool for genetic analysis," *Reviews-Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, FCTRX protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium

chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding FCTR<sub>X</sub> or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) FCTR<sub>X</sub> protein. Accordingly, the invention further provides methods for producing FCTR<sub>X</sub> protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding FCTR<sub>X</sub> protein has been introduced) in a suitable medium such that FCTR<sub>X</sub> protein is produced. In another embodiment, the method further comprises isolating FCTR<sub>X</sub> protein from the medium or the host cell.

### Transgenic FCTR<sub>X</sub> Animals

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which FCTR<sub>X</sub> protein-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous FCTR<sub>X</sub> sequences have been introduced into their genome or homologous recombinant animals in which endogenous FCTR<sub>X</sub> sequences have been altered. Such animals are useful for studying the function and/or activity of FCTR<sub>X</sub> protein and for identifying and/or evaluating modulators of FCTR<sub>X</sub> protein activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene

is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous FCTR<sub>X</sub> gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing FCTR<sub>X</sub>-encoding nucleic acid into the male pronuclei of a fertilized oocyte (*e.g.*, by microinjection, retroviral infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. The human FCTR<sub>X</sub> cDNA sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, can be introduced as a transgene into the genome of a non-human animal. Alternatively, a non-human homologue of the human FCTR<sub>X</sub> gene, such as a mouse FCTR<sub>X</sub> gene, can be isolated based on hybridization to the human FCTR<sub>X</sub> cDNA (described further *supra*) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably-linked to the FCTR<sub>X</sub> transgene to direct expression of FCTR<sub>X</sub> protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan, 1986. In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the FCTR<sub>X</sub> transgene in its genome and/or expression of FCTR<sub>X</sub> mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene-encoding FCTR<sub>X</sub> protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an FCTR<sub>X</sub> gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the FCTR<sub>X</sub> gene. The FCTR<sub>X</sub> gene can be a human gene (*e.g.*, the cDNA of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29), but more preferably, is a non-human homologue of a human FCTR<sub>X</sub> gene. For example, a mouse homologue of human FCTR<sub>X</sub> gene of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, can be used to construct a homologous recombination vector suitable for

altering an endogenous FCTR<sub>X</sub> gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous FCTR<sub>X</sub> gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous FCTR<sub>X</sub> gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous FCTR<sub>X</sub> protein). In the homologous recombination vector, the altered portion of the FCTR<sub>X</sub> gene is flanked at its 5'- and 3'-termini by additional nucleic acid of the FCTR<sub>X</sub> gene to allow for homologous recombination to occur between the exogenous FCTR<sub>X</sub> gene carried by the vector and an endogenous FCTR<sub>X</sub> gene in an embryonic stem cell. The additional flanking FCTR<sub>X</sub> nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5'- and 3'-termini) are included in the vector. *See, e.g.*, Thomas, *et al.*, 1987. *Cell* 51: 503 for a description of homologous recombination vectors. The vector is then introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced FCTR<sub>X</sub> gene has homologously-recombined with the endogenous FCTR<sub>X</sub> gene are selected. *See, e.g.*, Li, *et al.*, 1992. *Cell* 69: 915.

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. *See, e.g.*, Bradley, 1987. In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously-recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously-recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, 1991. *Curr. Opin. Biotechnol.* 2: 823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, *See, e.g.*, Lakso, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae*. *See, e.g.*, O'Gorman, *et al.*, 1991. *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing

transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, *et al.*, 1997. *Nature* 385: 810-813. In brief, a cell (*e.g.*, a somatic cell) from the transgenic animal can be isolated and induced to exit the growth cycle and enter G<sub>0</sub> phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell (*e.g.*, the somatic cell) is isolated.

### Pharmaceutical Compositions

The FCTR<sub>X</sub> nucleic acid molecules, FCTR<sub>X</sub> proteins, and anti-FCTR<sub>X</sub> antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral,



intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, an FCTR protein or anti-FCTR antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of

the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including

liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.*, U.S. Patent No. 5,328,470) or by stereotactic injection (*see, e.g.*, Chen, *et al.*, 1994. *Proc. Natl. Acad. Sci. USA* 91: 3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

### Screening and Detection Methods

The isolated nucleic acid molecules of the invention can be used to express FCTR<sub>X</sub> protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect FCTR<sub>X</sub> mRNA (*e.g.*, in a biological sample) or a genetic lesion in an FCTR<sub>X</sub> gene, and to modulate FCTR<sub>X</sub> activity, as described further, below. In addition, the FCTR<sub>X</sub> proteins can be used to screen drugs or compounds that modulate the FCTR<sub>X</sub> protein activity or expression as well as to treat disorders characterized by insufficient or excessive production of FCTR<sub>X</sub> protein or production of FCTR<sub>X</sub> protein forms that have decreased or aberrant activity compared to FCTR<sub>X</sub> wild-type protein (*e.g.*; diabetes (regulates insulin release); obesity (binds and transport lipids); metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers,

and infectious disease (possesses anti-microbial activity) and the various dyslipidemias. In addition, the anti-FCTR<sub>X</sub> antibodies of the invention can be used to detect and isolate FCTR<sub>X</sub> proteins and modulate FCTR<sub>X</sub> activity. In yet a further aspect, the invention can be used in methods to influence appetite, absorption of nutrients and the disposition of metabolic substrates in both a positive and negative fashion.

The invention further pertains to novel agents identified by the screening assays described herein and uses thereof for treatments as described, *supra*.

### Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to FCTR<sub>X</sub> proteins or have a stimulatory or inhibitory effect on, *e.g.*, FCTR<sub>X</sub> protein expression or FCTR<sub>X</sub> protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of an FCTR<sub>X</sub> protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g.*, Lam, 1997. *Anticancer Drug Design* 12: 145.

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678; Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (e.g., Houghten, 1992.

*Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor, 1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner, U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science* 249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of FCTR protein, or a biologically-active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to an FCTR protein determined. The cell, for example, can of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the FCTR protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the FCTR protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with  $^{125}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , or  $^3\text{H}$ , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of FCTR protein, or a biologically-active portion thereof, on the cell surface with a known compound which binds FCTR to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an FCTR protein, wherein determining the ability of the test compound to interact with an FCTR protein comprises determining the ability of the test compound to preferentially bind to FCTR protein or a biologically-active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of FCTR protein, or a biologically-active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the FCTR protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of FCTR or a biologically-active portion thereof can be accomplished, for example, by determining the ability of the FCTR protein to bind to or interact with an FCTR target molecule. As used herein, a "target molecule" is a molecule with which an FCTR protein

binds or interacts in nature, for example, a molecule on the surface of a cell which expresses an FCTR<sub>X</sub> interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. An FCTR<sub>X</sub> target molecule can be a non-FCTR<sub>X</sub> molecule or an FCTR<sub>X</sub> protein or polypeptide of the invention. In one embodiment, an FCTR<sub>X</sub> target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (e.g. a signal generated by binding of a compound to a membrane-bound FCTR<sub>X</sub> molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with FCTR<sub>X</sub>.

Determining the ability of the FCTR<sub>X</sub> protein to bind to or interact with an FCTR<sub>X</sub> target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the FCTR<sub>X</sub> protein to bind to or interact with an FCTR<sub>X</sub> target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca<sup>2+</sup>, diacylglycerol, IP<sub>3</sub>, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising an FCTR<sub>X</sub>-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting an FCTR<sub>X</sub> protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to bind to the FCTR<sub>X</sub> protein or biologically-active portion thereof. Binding of the test compound to the FCTR<sub>X</sub> protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the FCTR<sub>X</sub> protein or biologically-active portion thereof with a known compound which binds FCTR<sub>X</sub> to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an FCTR<sub>X</sub> protein, wherein determining the ability of the test compound to interact with an FCTR<sub>X</sub> protein comprises determining the ability of the test compound to preferentially bind to FCTR<sub>X</sub> or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting FCTR<sub>X</sub> protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the FCTR<sub>X</sub> protein or biologically-active portion thereof. Determining the ability of the test compound to modulate

the activity of FCTR<sub>X</sub> can be accomplished, for example, by determining the ability of the FCTR<sub>X</sub> protein to bind to an FCTR<sub>X</sub> target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of FCTR<sub>X</sub> protein can be accomplished by determining the ability of the FCTR<sub>X</sub> protein further modulate an FCTR<sub>X</sub> target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described, *supra*.

In yet another embodiment, the cell-free assay comprises contacting the FCTR<sub>X</sub> protein or biologically-active portion thereof with a known compound which binds FCTR<sub>X</sub> protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an FCTR<sub>X</sub> protein, wherein determining the ability of the test compound to interact with an FCTR<sub>X</sub> protein comprises determining the ability of the FCTR<sub>X</sub> protein to preferentially bind to or modulate the activity of an FCTR<sub>X</sub> target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of FCTR<sub>X</sub> protein. In the case of cell-free assays comprising the membrane-bound form of FCTR<sub>X</sub> protein, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of FCTR<sub>X</sub> protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton<sup>®</sup> X-100, Triton<sup>®</sup> X-114, Thesit<sup>®</sup>, Isotridecypoly(ethylene glycol ether)<sub>n</sub>, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either FCTR<sub>X</sub> protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to FCTR<sub>X</sub> protein, or interaction of FCTR<sub>X</sub> protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-FCTR<sub>X</sub> fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or FCTR<sub>X</sub> protein, and the mixture is

incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of FCTR<sub>X</sub> protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the FCTR<sub>X</sub> protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated FCTR<sub>X</sub> protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with FCTR<sub>X</sub> protein or target molecules, but which do not interfere with binding of the FCTR<sub>X</sub> protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or FCTR<sub>X</sub> protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the FCTR<sub>X</sub> protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the FCTR<sub>X</sub> protein or target molecule.

In another embodiment, modulators of FCTR<sub>X</sub> protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of FCTR<sub>X</sub> mRNA or protein in the cell is determined. The level of expression of FCTR<sub>X</sub> mRNA or protein in the presence of the candidate compound is compared to the level of expression of FCTR<sub>X</sub> mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of FCTR<sub>X</sub> mRNA or protein expression based upon this comparison. For example, when expression of FCTR<sub>X</sub> mRNA or protein is greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of FCTR<sub>X</sub> mRNA or protein expression. Alternatively, when expression of FCTR<sub>X</sub> mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of FCTR<sub>X</sub> mRNA or protein expression. The level of FCTR<sub>X</sub> mRNA or protein expression in the cells can be determined by methods described herein for detecting FCTR<sub>X</sub> mRNA or protein.

In yet another aspect of the invention, the FCTR<sub>X</sub> proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*,



1993. *Cell* 72: 223-232; Madura, *et al.*, 1993. *J. Biol. Chem.* 268: 12046-12054; Bartel, *et al.*, 1993. *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993. *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with FCTR<sub>X</sub> ("FCTR<sub>X</sub>-binding proteins" or "FCTR<sub>X</sub>-bp") and modulate FCTR<sub>X</sub> activity. Such FCTR<sub>X</sub>-binding proteins are also likely to be involved in the propagation of signals by the FCTR<sub>X</sub> proteins as, for example, upstream or downstream elements of the FCTR<sub>X</sub> pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for FCTR<sub>X</sub> is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming an FCTR<sub>X</sub>-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with FCTR<sub>X</sub>.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof for treatments as described herein.

### **Detection Assays**

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. By way of example, and not of limitation, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. Some of these applications are described in the subsections, below.

### **Chromosome Mapping**

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the FCTR<sub>X</sub> sequences, SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or fragments or derivatives thereof, can be used to map the location of the FCTR<sub>X</sub> genes, respectively, on a chromosome. The

mapping of the FCTR<sub>X</sub> sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, FCTR<sub>X</sub> genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the FCTR<sub>X</sub> sequences. Computer analysis of the FCTR<sub>X</sub> sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the FCTR<sub>X</sub> sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. See, e.g., D'Eustachio, *et al.*, 1983. *Science* 220: 919-924. Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the FCTR<sub>X</sub> sequences to design oligonucleotide primers, sub-localization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a

review of this technique, *see*, Verma, *et al.*, HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, *e.g.*, in McKusick, MENDELIAN INHERITANCE IN MAN, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, *e.g.*, Egeland, *et al.*, 1987. *Nature*, 325: 783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the FCTR<sub>X</sub> gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

### **Tissue Typing**

The FCTR<sub>X</sub> sequences of the invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Patent No. 5,272,057).

Furthermore, the sequences of the invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the FCTR<sub>X</sub> sequences described herein can be used to prepare two PCR primers from the 5'- and 3'-termini of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the invention can be used to obtain such identification sequences from individuals and from tissue. The FCTR<sub>X</sub> sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

#### **Predictive Medicine**

The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the invention relates to diagnostic assays for determining FCTR<sub>X</sub> protein and/or nucleic acid expression as well as FCTR<sub>X</sub> activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant FCTR<sub>X</sub> expression or activity. The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with FCTR<sub>X</sub> protein, nucleic acid expression or activity. For example, mutations in an FCTR<sub>X</sub> gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior

to the onset of a disorder characterized by or associated with FCTR<sub>X</sub> protein, nucleic acid expression, or biological activity.

Another aspect of the invention provides methods for determining FCTR<sub>X</sub> protein, nucleic acid expression or activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of FCTR<sub>X</sub> in clinical trials.

These and other agents are described in further detail in the following sections.

#### *Diagnostic Assays*

An exemplary method for detecting the presence or absence of FCTR<sub>X</sub> in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting FCTR<sub>X</sub> protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes FCTR<sub>X</sub> protein such that the presence of FCTR<sub>X</sub> is detected in the biological sample. An agent for detecting FCTR<sub>X</sub> mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to FCTR<sub>X</sub> mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length FCTR<sub>X</sub> nucleic acid, such as the nucleic acid of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to FCTR<sub>X</sub> mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting FCTR<sub>X</sub> protein is an antibody capable of binding to FCTR<sub>X</sub> protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method

of the invention can be used to detect FCTR<sub>X</sub> mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of FCTR<sub>X</sub> mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of FCTR<sub>X</sub> protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of FCTR<sub>X</sub> genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of FCTR<sub>X</sub> protein include introducing into a subject a labeled anti-FCTR<sub>X</sub> antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting FCTR<sub>X</sub> protein, mRNA, or genomic DNA, such that the presence of FCTR<sub>X</sub> protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of FCTR<sub>X</sub> protein, mRNA or genomic DNA in the control sample with the presence of FCTR<sub>X</sub> protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of FCTR<sub>X</sub> in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting FCTR<sub>X</sub> protein or mRNA in a biological sample; means for determining the amount of FCTR<sub>X</sub> in the sample; and means for comparing the amount of FCTR<sub>X</sub> in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect FCTR<sub>X</sub> protein or nucleic acid.

#### *Prognostic Assays*

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant FCTR<sub>X</sub> expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with FCTR<sub>X</sub> protein, nucleic acid expression or activity. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the invention provides a method for identifying a disease or disorder associated with aberrant FCTR<sub>X</sub> expression or activity in which a test sample is obtained from a subject and FCTR<sub>X</sub> protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein

the presence of FCTR<sub>X</sub> protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant FCTR<sub>X</sub> expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant FCTR<sub>X</sub> expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder. Thus, the invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant FCTR<sub>X</sub> expression or activity in which a test sample is obtained and FCTR<sub>X</sub> protein or nucleic acid is detected (e.g., wherein the presence of FCTR<sub>X</sub> protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant FCTR<sub>X</sub> expression or activity).

The methods of the invention can also be used to detect genetic lesions in an FCTR<sub>X</sub> gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding an FCTR<sub>X</sub>-protein, or the misexpression of the FCTR<sub>X</sub> gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of: (i) a deletion of one or more nucleotides from an FCTR<sub>X</sub> gene; (ii) an addition of one or more nucleotides to an FCTR<sub>X</sub> gene; (iii) a substitution of one or more nucleotides of an FCTR<sub>X</sub> gene, (iv) a chromosomal rearrangement of an FCTR<sub>X</sub> gene; (v) an alteration in the level of a messenger RNA transcript of an FCTR<sub>X</sub> gene, (vi) aberrant modification of an FCTR<sub>X</sub> gene, such as of the methylation pattern of the genomic DNA, (vii) the presence of a non-wild-type splicing pattern of a messenger RNA transcript of an FCTR<sub>X</sub> gene, (viii) a non-wild-type level of an FCTR<sub>X</sub> protein, (ix) allelic loss of an FCTR<sub>X</sub> gene, and (x) inappropriate post-translational modification of an FCTR<sub>X</sub> protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in an FCTR<sub>X</sub> gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202), such as

anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g., Landegran, et al., 1988. Science 241: 1077-1080; and Nakazawa, et al., 1994. Proc. Natl. Acad. Sci. USA 91: 360-364*), the latter of which can be particularly useful for detecting point mutations in the FCTR<sub>X</sub>-gene (*see, Abravaya, et al., 1995. Nucl. Acids Res. 23: 675-682*). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g., genomic, mRNA or both*) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to an FCTR<sub>X</sub> gene under conditions such that hybridization and amplification of the FCTR<sub>X</sub> gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (*see, Guatelli, et al., 1990. Proc. Natl. Acad. Sci. USA 87: 1874-1878*), transcriptional amplification system (*see, Kwoh, et al., 1989. Proc. Natl. Acad. Sci. USA 86: 1173-1177*); Q $\beta$  Replicase (*see, Lizardi, et al, 1988. BioTechnology 6: 1197*), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an FCTR<sub>X</sub> gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g., U.S. Patent No. 5,493,531*) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in FCTR<sub>X</sub> can be identified by hybridizing a sample and control nucleic acids, *e.g., DNA or RNA*, to high-density arrays containing hundreds or thousands of oligonucleotides probes. *See, e.g., Cronin, et al., 1996. Human Mutation 7: 244-255; Kozal, et al., 1996. Nat. Med. 2: 753-759*. For example, genetic mutations in FCTR<sub>X</sub> can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, *et al., supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of



point mutations. This is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the FCTR<sub>X</sub> gene and detect mutations by comparing the sequence of the sample FCTR<sub>X</sub> with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert, 1977. *Proc. Natl. Acad. Sci. USA* 74: 560 or Sanger, 1977. *Proc. Natl. Acad. Sci. USA* 74: 5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (see, e.g., Naeve, et al., 1995. *Biotechniques* 19: 448), including sequencing by mass spectrometry (see, e.g., PCT International Publication No. WO 94/16101; Cohen, et al., 1996. *Adv. Chromatography* 36: 127-162; and Griffin, et al., 1993. *Appl. Biochem. Biotechnol.* 38: 147-159).

Other methods for detecting mutations in the FCTR<sub>X</sub> gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes. See, e.g., Myers, et al., 1985. *Science* 230: 1242. In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type FCTR<sub>X</sub> sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S<sub>1</sub> nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, e.g., Cotton, et al., 1988. *Proc. Natl. Acad. Sci. USA* 85: 4397; Saleeba, et al., 1992. *Methods Enzymol.* 217: 286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in FCTR<sub>X</sub> cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at

G/T mismatches. *See, e.g., Hsu, et al., 1994. Carcinogenesis* 15: 1657-1662. According to an exemplary embodiment, a probe based on an FCTR sequence, *e.g.,* a wild-type FCTR sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See, e.g., U.S. Patent No. 5,459,039.*

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in FCTR genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids. *See, e.g., Orita, et al., 1989. Proc. Natl. Acad. Sci. USA: 86: 2766; Cotton, 1993. Mutat. Res. 285: 125-144; Hayashi, 1992. Genet. Anal. Tech. Appl. 9: 73-79.* Single-stranded DNA fragments of sample and control FCTR nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. *See, e.g., Keen, et al., 1991. Trends Genet. 7: 5.*

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). *See, e.g., Myers, et al., 1985. Nature* 313: 495. When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. *See, e.g., Rosenbaum and Reissner, 1987. Biophys. Chem. 265: 12753.*

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. *See, e.g., Saiki, et al., 1986. Nature* 324: 163; Saiki, et al., 1989. *Proc. Natl. Acad. Sci. USA* 86: 6230. Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization; *see, e.g.,* Gibbs, *et al.*, 1989. *Nucl. Acids Res.* 17: 2437-2448) or at the extreme 3'-terminus of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (*see, e.g.,* Prossner, 1993. *Tibtech.* 11: 238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. *See, e.g.,* Gasparini, *et al.*, 1992. *Mol. Cell Probes* 6: 1. It is anticipated that in certain embodiments amplification may also be performed using *Taq* ligase for amplification. *See, e.g.,* Barany, 1991. *Proc. Natl. Acad. Sci. USA* 88: 189. In such cases, ligation will occur only if there is a perfect match at the 3'-terminus of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.,* in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an FCTR<sub>X</sub> gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which FCTR<sub>X</sub> is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

#### *Pharmacogenomics*

Agents, or modulators that have a stimulatory or inhibitory effect on FCTR<sub>X</sub> activity (*e.g.,* FCTR<sub>X</sub> gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.) In conjunction with such treatment, the pharmacogenomics (*i.e.,* the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.,*

drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of FCTRX protein, expression of FCTRX nucleic acid, or mutation content of FCTRX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, 1996. *Clin. Exp. Pharmacol. Physiol.*, 23: 983-985; Linder, 1997. *Clin. Chem.*, 43: 254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is hemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of FCTRX protein, expression of FCTRX nucleic acid, or mutation content of FCTRX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition,

pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an FCTR<sub>X</sub> modulator, such as a modulator identified by one of the exemplary screening assays described herein.

#### *Monitoring of Effects During Clinical Trials*

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of FCTR<sub>X</sub> (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase FCTR<sub>X</sub> gene expression, protein levels, or upregulate FCTR<sub>X</sub> activity, can be monitored in clinical trials of subjects exhibiting decreased FCTR<sub>X</sub> gene expression, protein levels, or downregulated FCTR<sub>X</sub> activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease FCTR<sub>X</sub> gene expression, protein levels, or downregulate FCTR<sub>X</sub> activity, can be monitored in clinical trials of subjects exhibiting increased FCTR<sub>X</sub> gene expression, protein levels, or upregulated FCTR<sub>X</sub> activity. In such clinical trials, the expression or activity of FCTR<sub>X</sub> and, preferably, other genes that have been implicated in, for example, a cellular proliferation or immune disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell.

By way of example, and not of limitation, genes, including FCTR<sub>X</sub>, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates FCTR<sub>X</sub> activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of FCTR<sub>X</sub> and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of FCTR<sub>X</sub> or other genes. In this manner, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the

screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of an FCTR<sub>X</sub> protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the FCTR<sub>X</sub> protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the FCTR<sub>X</sub> protein, mRNA, or genomic DNA in the pre-administration sample with the FCTR<sub>X</sub> protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of FCTR<sub>X</sub> to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of FCTR<sub>X</sub> to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

## Methods of Treatment

The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant FCTR<sub>X</sub> expression or activity. The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers. These methods of treatment will be discussed more fully, below.

### *Disease and Disorders*

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to: (i) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (ii) antibodies to an aforementioned peptide; (iii) nucleic acids encoding an aforementioned peptide; (iv) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) that are utilized to "knockout" endogenous function of an aforementioned peptide by homologous recombination (*see, e.g.*, Capecchi, 1989. *Science* 244: 1288-1292); or (v) modulators (*i.e.*, inhibitors, agonists and antagonists, including

additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with

5 Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or  
10 RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.)  
15 and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, and the like).

#### *Prophylactic Methods*

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant FCTR<sub>X</sub> expression or activity, by administering to the  
20 subject an agent that modulates FCTR<sub>X</sub> expression or at least one FCTR<sub>X</sub> activity. Subjects at risk for a disease that is caused or contributed to by aberrant FCTR<sub>X</sub> expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the FCTR<sub>X</sub> aberrancy, such that a disease or disorder is prevented  
25 or, alternatively, delayed in its progression. Depending upon the type of FCTR<sub>X</sub> aberrancy, for example, an FCTR<sub>X</sub> agonist or FCTR<sub>X</sub> antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the invention are further discussed in the following subsections.

#### *Therapeutic Methods*

30 Another aspect of the invention pertains to methods of modulating FCTR<sub>X</sub> expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of FCTR<sub>X</sub> protein activity associated with the cell. An agent that modulates FCTR<sub>X</sub> protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of an  
35 FCTR<sub>X</sub> protein, a peptide, an FCTR<sub>X</sub> peptidomimetic, or other small molecule. In one

embodiment, the agent stimulates one or more FCTR<sub>X</sub> protein activity. Examples of such stimulatory agents include active FCTR<sub>X</sub> protein and a nucleic acid molecule encoding FCTR<sub>X</sub> that has been introduced into the cell. In another embodiment, the agent inhibits one or more FCTR<sub>X</sub> protein activity. Examples of such inhibitory agents include antisense FCTR<sub>X</sub> nucleic acid molecules and anti-FCTR<sub>X</sub> antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of an FCTR<sub>X</sub> protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., up-regulates or down-regulates) FCTR<sub>X</sub> expression or activity. In another embodiment, the method involves administering an FCTR<sub>X</sub> protein or nucleic acid molecule as therapy to compensate for reduced or aberrant FCTR<sub>X</sub> expression or activity.

Stimulation of FCTR<sub>X</sub> activity is desirable in situations in which FCTR<sub>X</sub> is abnormally downregulated and/or in which increased FCTR<sub>X</sub> activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and/or differentiation (e.g., cancer or immune associated disorders). Another example of such a situation is where the subject has a gestational disease (e.g., preclampsia).

### Determination of the Biological Effect of the Therapeutic

In various embodiments of the invention, suitable *in vitro* or *in vivo* assays are performed to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

### Prophylactic and Therapeutic Uses of the Compositions of the Invention

The FCTR<sub>X</sub> nucleic acids and proteins of the invention are useful in potential prophylactic and therapeutic applications implicated in a variety of disorders including, but not limited to: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias, metabolic disturbances



associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.

As an example, a cDNA encoding the FCTR<sub>X</sub> protein of the invention may be useful in gene therapy, and the protein may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the invention will have efficacy for treatment of patients suffering from: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias.

Both the novel nucleic acid encoding the FCTR<sub>X</sub> protein, and the FCTR<sub>X</sub> protein of the invention, or fragments thereof, may also be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. A further use could be as an anti-bacterial molecule (*i.e.*, some peptides have been found to possess anti-bacterial properties). These materials are further useful in the generation of antibodies which immunospecifically-bind to the novel substances of the invention for use in therapeutic or diagnostic methods.

## EXAMPLES

The following examples illustrate by way of non-limiting example various aspects of the invention.

### Example 1: Method of Identifying the Nucleic Acids

The novel nucleic acids of the invention were identified by TblastN using CuraGen Corporation's sequence file, run against the Genomic Daily Files made available by GenBank. The nucleic acids were further predicted by the program GenScan™, including selection of exons. These were further modified by means of similarities using BLAST searches. The sequences were then manually corrected for apparent inconsistencies, thereby obtaining the sequences encoding the full-length proteins.

### Example 2. Quantitative expression analysis of FCTR2 in various cells and tissues

The quantitative expression of clone AL078594\_A (FCTR2) was assessed in a large number of normal and tumor sample cells and cell lines (Panel 1), as well as in surgical tissue samples (Panel 2), by real time quantitative PCR (TAQMAN®) performed on a Perkin-Elmer Biosystems ABI PRISM® 7700 Sequence Detection System.

First, 96 RNA samples were normalized to  $\beta$ -actin and GAPDH. RNA (~50 ng total or ~1 ng polyA+) was converted to cDNA using the TAQMAN® Reverse Transcription Reagents Kit (PE Biosystems, Foster City, CA; Catalog No. N808-0234) and random hexamers according to the manufacturer's protocol. Reactions were performed in 20  $\mu$ l and incubated for 30 min. at 48°C. cDNA (5  $\mu$ l) was then transferred to a separate plate for the TAQMAN® reaction using -actin and GAPDH TAQMAN® Assay Reagents (PE Biosystems; Catalog Nos. 4310881E and 4310884E, respectively) and TAQMAN® universal PCR Master Mix (PE Biosystems; Catalog No. 4304447) according to the manufacturer's protocol. Reactions were performed in 25  $\mu$ l using the following parameters: 2 min. at 50°C; 10 min. at 95°C; 15 sec. at 95°C/1 min. at 60°C (40 cycles). Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100. The average CT values obtained for  $\beta$ -actin and GAPDH were used to normalize RNA samples. The RNA sample generating the highest CT value required no further diluting, while all other samples were diluted relative to this sample according to their  $\beta$ -actin /GAPDH average CT values.

Normalized RNA (5  $\mu$ l) was converted to cDNA and analyzed via TAQMAN® using One Step RT-PCR Master Mix Reagents (PE Biosystems; Catalog No. 4309169) and gene-specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's *Primer Express* Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature ( $T_m$ ) range = 58°-60° C, primer optimal  $T_m$  = 59° C, maximum primer difference = 2° C, probe does not have 5' G, probe  $T_m$  must be 10° C greater than primer  $T_m$ , amplicon size 75 bp to 100 bp. The probes and primers selected (see below) were synthesized by Synthegen (Houston, TX, USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900 nM each, and probe, 200nM.

The expression was probed with the primer-probe set Ag 259. The Forward primer sequence is 5'-GGAGAGGCTCTGAAGCTACACAA-3' (SEQ ID NO:31); the Probe primer sequence is TET-5'-TCAGCTGCACAAGCCCCCTGCT-3'-TAMRA (SEQ ID NO:32); and the

Reverse primer sequence is 5'-GCAGTGGTTGGAGCTGGAA-3' (SEQ ID NO:33). Table 15 shows the primer locations within the FCTR2 nucleic acid sequence.

Table 15. Primer-Probe Set Ag259

Primers	Length	Start Position
Forward	23	124
Probe	22	158
Reverse	19	181

PCR conditions: Normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate (Perkin Elmer Biosystems). PCR cocktails including two probes (a probe specific for the target clone and another gene-specific probe multiplexed with the target probe) were set up using 1X TaqMan™ PCR Master Mix for the PE Biosystems 7700, with 5 mM MgCl<sub>2</sub>, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold™ (PE Biosystems), and 0.4 U/μl RNase inhibitor, and 0.25 U/μl reverse transcriptase. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute.

The results for various cells and cell lines that constitute Panel 1 are shown in Table 16. In Table 16, the following abbreviations are used: ca. = carcinoma; \* = established from metastasis; met = metastasis; s cell var= small cell variant; non-s = non-sm =non-small; squam = squamous; pl. eff = pl effusion = pleural effusion; glio = glioma; astro = astrocytoma; and neuro = neuroblastoma.

Table 16.

Tissue Name	Rel. Expr., %	Tissue Name	Rel. Expr., %
Adipose	100.0	Colon ca. HT29	0.2
Adrenal gland	0.0	Colon ca. CaCo-2	0.0
Bladder	0.2	Colon ca. HCT-15	3.0
Bone marrow	0.0	Colon ca. HCT-116	0.0
Endothelial cells	0.0	Colon ca. HCC-2998	0.2
Endothelial cells (treated)	0.0	Colon ca. SW480	1.5
Liver	1.5	Colon ca.* (SW480 met)SW620	0.0
Liver (fetal)	0.0	Fetal Skeletal	0.3
Spleen	0.0	Skeletal muscle	2.6
Thymus	0.0	Heart	6.4
Thyroid	0.0	Stomach	0.0
Trachea	0.0	Gastric ca.* (liver met) NCI-N87	0.3
Testis	0.1	Kidney	4.0
Spinal cord	0.6	Kidney (fetal)	0.1
Salavary gland	0.0	Renal ca. 786-0	0.0

Brain (amygdala)	0.0	Renal ca. A498	0.1
Brain (cerebellum)	2.9	Renal ca. ACHN	0.0
Brain (hippocampus)	0.0	Renal ca. TK-10	0.1
Brain (substantia nigra)	4.8	Renal ca. UO-31	0.1
Brain (thalamus)	0.1	Renal ca. RXF 393	0.0
Cerebral Cortex	0.0	Pancreas	1.5
Brain (whole)	0.0	Pancreatic ca. CAPAN 2	0.2
Brain (fetal)	0.0	Ovary	0.2
CNS ca. (glio/astro) U-118-MG	0.2	Ovarian ca. IGROV-1	0.7
CNS ca. (astro) SF-539	0.0	Ovarian ca. OVCAR-3	51.1
CNS ca. (astro) SNB-75	0.0	Ovarian ca. OVCAR-4	52.9
CNS ca. (astro) SW1783	0.0	Ovarian ca. OVCAR-5	21.6
CNS ca. (glio) U251	0.2	Ovarian ca. OVCAR-8	0.2
CNS ca. (glio) SF-295	0.0	Ovarian ca.* (ascites) SK-OV-3	0.0
CNS ca. (glio) SNB-19	3.3	Prostate	0.0
CNS ca. (glio/astro) U87-MG	0.0	Prostate ca.* (bone met)PC-3	0.0
CNS ca.* (neuro; met ) SK-N-AS	0.0	Placenta	0.0
Small intestine	0.1	Pituitary gland	0.5
Colorectal	0.1	Uterus	0.0

It is seen from Table 16 that there is high expression of sequence AL078594\_A found in several ovarian cancer cell lines, and very high expression in normal adipose tissue.

## Panel 2

Panel 2 consists of a 96 well plate (2 control wells, 94 test samples) composed of RNA/cDNA isolated from human tissue procured by surgeons working in close cooperation with the National Cancer Institute's Cooperative Human Tissue Network (CHTN) or the National Disease Research Initiative (NDRI). The tissues procured are derived from human malignancies and in cases where indicated many malignant tissues have "matched margins". The tumor tissue and the "matched margins" are evaluated by two independent pathologists (the surgical pathologists and again by a pathologists at NDRI or CHTN). This analysis provides a gross histopathological assessment of tumor differentiation grade. Moreover, most samples include the original surgical pathology report that provides information regarding the clinical stage of the patient. These matched margins are taken from the tissue surrounding (i.e. immediately proximal) to the zone of surgery (designated "NAT", for normal adjacent tissue, in Table 17). In addition, RNA/cDNA was obtained from various human tissues derived from human autopsies performed on deceased elderly people or sudden death victims (accidents, etc.).

These tissue were ascertained to be free of disease and were purchased from various high quality commercial sources such as Clontech, Research Genetics, and Invitrogen.

RNA integrity from all samples is controlled for quality by visual assessment of agarose gel electrophoresis using 28s and 18s ribosomal RNA staining intensity ratio as a guide (2:1 to 2.5:1 28s:18s) and the presence of low molecular weight RNAs indicative of degradation products. Samples are quality controlled for genomic DNA contamination by reactions run in the absence of reverse transcriptase using probe and primer sets designed to amplify across the span of a single exon.

**Table 17.**

<b>Tissue Name</b>	<b>Rel. Expr. %</b>	<b>Tissue Name</b>	<b>Rel. Expr. %</b>
Normal Colon GENPAK 061003	0.0	Kidney Cancer Clontech 8120607	0.0
83219 CC Well to Mod Diff (ODO3866)	0.0	Kidney NAT Clontech 8120608	0.0
83220 CC NAT (ODO3866)	0.0	Kidney Cancer Clontech 8120613	0.0
83221 CC Gr.2 rectosigmoid (ODO3868)	0.0	Kidney NAT Clontech 8120614	0.0
83222 CC NAT (ODO3868)	0.0	Kidney Cancer Clontech 9010320	0.0
83235 CC Mod Diff (ODO3920)	0.0	Kidney NAT Clontech 9010321	0.0
83236 CC NAT (ODO3920)	0.0	Normal Uterus GENPAK 061018	0.0
83237 CC Gr.2 ascend colon (ODO3921)	0.0	Uterus Cancer GENPAK 064011	0.0
83238 CC NAT (ODO3921)	0.0	Normal Thyroid Clontech A+ 6570-1**	0.0
83241 CC from Partial Hepatectomy (ODO4309)	0.0	Thyroid Cancer GENPAK 064010	0.0
83242 Liver NAT (ODO4309)	0.0	Thyroid Cancer INVITROGEN A302152	0.0
87472 Colon mets to lung (OD04451-01)	0.0	Thyroid NAT INVITROGEN A302153	0.0
87473 Lung NAT (OD04451-02)	0.0	Normal Breast GENPAK 061019	0.0
Normal Prostate Clontech A+ 6546-1	0.0	84877 Breast Cancer (OD04566)	0.0
84140 Prostate Cancer (OD04410)	0.0	85975 Breast Cancer (OD04590-01)	0.0
84141 Prostate NAT (OD04410)	0.0	85976 Breast Cancer Mets (OD04590-03)	0.0
87073 Prostate Cancer (OD04720-01)	0.0	87070 Breast Cancer Metastasis (OD04655-05)	0.0
87074 Prostate NAT (OD04720-02)	0.0	GENPAK Breast Cancer 064006	0.0
Normal Lung GENPAK 061010	0.0	Breast Cancer Clontech 9100266	34.6
83239 Lung Met to Muscle (ODO4286)	0.0	Breast NAT Clontech 9100265	100.0
83240 Muscle NAT (ODO4286)	0.0	Breast Cancer INVITROGEN	0.0

		A209073	
84136 Lung Malignant Cancer (OD03126)	0.1	Breast NAT INVITROGEN A2090734	0.0
84137 Lung NAT (OD03126)	0.0	Normal Liver GENPAK 061009	0.0
84871 Lung Cancer (OD04404)	0.0	Liver Cancer GENPAK 064003	0.0
84872 Lung NAT (OD04404)	0.0	Liver Cancer Research Genetics RNA 1025	0.0
84875 Lung Cancer (OD04565)	0.0	Liver Cancer Research Genetics RNA 1026	0.0
85950 Lung Cancer (OD04237-01)	0.2	Paired Liver Cancer Tissue Research Genetics RNA 6004-T	0.0
85970 Lung NAT (OD04237-02)	0.0	Paired Liver Tissue Research Genetics RNA 6004-N	0.0
83255 Ocular Mel Met to Liver (ODO4310)	0.0	Paired Liver Cancer Tissue Research Genetics RNA 6005-T	0.0
83256 Liver NAT (ODO4310)	0.0	Paired Liver Tissue Research Genetics RNA 6005-N	0.0
84139 Melanoma Mets to Lung (OD04321)	0.0	Normal Bladder GENPAK 061001	0.0
84138 Lung NAT (OD04321)	0.0	Bladder Cancer Research Genetics RNA 1023	0.3
Normal Kidney GENPAK 061008	0.0	Bladder Cancer INVITROGEN A302173	0.0
83786 Kidney Ca, Nuclear grade 2 (OD04338)	0.0	87071 Bladder Cancer (OD04718-01)	0.0
83787 Kidney NAT (OD04338)	0.0	87072 Bladder Normal Adjacent (OD04718-03)	0.0
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	Normal Ovary Res. Gen.	0.0
83789 Kidney NAT (OD04339)	0.0	Ovarian Cancer GENPAK 064008	0.0
83790 Kidney Ca, Clear cell type (OD04340)	0.0	87492 Ovary Cancer (OD04768-07)	0.0
83791 Kidney NAT (OD04340)	0.0	87493 Ovary NAT (OD04768-08)	0.0
83792 Kidney Ca, Nuclear grade 3 (OD04348)	0.0	Normal Stomach GENPAK 061017	0.0
83793 Kidney NAT (OD04348)	0.0	NAT Stomach Clontech 9060359	0.0
87474 Kidney Cancer (OD04622-01)	0.0	Gastric Cancer Clontech 9060395	0.0
87475 Kidney NAT (OD04622-03)	0.0	NAT Stomach Clontech 9060394	0.0
85973 Kidney Cancer (OD04450-01)	0.0	Gastric Cancer Clontech 9060397	0.0
85974 Kidney NAT (OD04450-03)	0.0	NAT Stomach Clontech 9060396	0.5
		Gastric Cancer GENPAK 064005	0.2

There is high expression of sequence AL078594\_A found in normal adjacent breast tissue and in breast cancer tissue. Panel 2 includes only two ovarian cancer samples, neither of which express this sequence.

- 5 Therefore, the FCTR2 protein of clone AL078594\_A may serve as the target for a diagnostic assay in certain ovarian cancers, and as a potential therapeutic target for this subset of ovarian cancer and possibly for breast cancer.

The citation of any reference herein should not be deemed as an admission that such reference is available as prior art to the instant invention.

### EQUIVALENTS

- 10 Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims which follow. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the claims.
- 15 The choice of nucleic acid starting material, clone of interest, or library type is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. Other aspects, advantages, and modifications considered to be within the scope of the following claims.